SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS

Dissertation submitted for

M.D. BIOCHEMISTRY BRANCH – XIII

DEGREE EXAMINATION

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI – 600 032

TAMILNADU

APRIL 2016

BONAFIDE CERTIFICATE

This to certify that this dissertation work entitled "**SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS"** is the original bonafide work done by **Dr.S.MICHAEL RAJAM GEETHA**, Post Graduate Student, Institute of Biochemistry, Madras Medical College, Chennai under our direct supervision and guidance.

Prof. Dr.V.K.Ramadesikan, MD., (Guide)
Professor,
Institute of Biochemistry
Madras Medical College
Chennai-600 003.

Prof. Dr. K.Ramadevi. MD.,
Director & Professor,
Institute of Biochemistry
Madras Medical College
Chennai-600 003.

Dean
Madras Medical College and
Rajiv Gandhi Government General Hospital,
Chennai - 600 003.

DECLARATION

I, **Dr.S.MICHAEL RAJAM GEETHA**, Post Graduate, Institute of Biochemistry, Madras Medical College, solemnly declare that the dissertation titled "**SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS"** is the bonafide work done by me at Institute of Biochemistry, Madras Medical College under the expert guidance and supervision of **Prof.Dr.V.K.RAMADESIKAN,** M.D., Professor, Institute of Biochemistry, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch XIII) in Biochemistry.

Place: Chennai

Date: **Dr. S.MICHAEL RAJAM GEETHA**

SPECIAL ACKNOWLEDGEMENT

The author gratefully acknowledges and sincerely thanks Professor **Dr.R.Vimala, M.D.,** Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai, for granting her permission to utilize the facilities of this Institution for the study.

ACKNOWLEDGEMENT

The author expresses her warmest respects and profound gratitude to Dr.K.Ramadevi,M.D., Director and Professor, Institute of Biochemistry, Madras Medical College, Chennai, for her academic enthusiasm and for facilitating her research work in the institute.

The author expresses her heartfelt gratitude to her guide and supervisor Dr.V.K. RAMADESIKAN,M.D., Professor, Institute of Biochemistry, Madras Medical College, Chennai, for his intellectual and valuable guidance, unfailing support, encouragement and continuous inspiration throughout the period of her study.

The author in particular, is extremely thankful to Dr.RAJESWARI,MD,DM, Professor and Head of the Department of Rheumatology, Government General Hospital,Chennai, for granting permission to obtain blood samples from the patients.

The author expresses her thanks to the Professors Dr.R.Chitraa,M.D, Dr.K.Pramila M.D, Dr.V.Amuthavalli,M.D, and Dr.Periyandavar,M.D., Institute of Biochemistry, Madras Medical College, for their guidance, encouragement, insightful comments and suggestions.

The author expresses her warm respects and sincere thanks to her co-guide. Dr.V.G. Karpaghavalli,M.D Assistant Professor, Institute of Biochemistry, Madras Medical College for her guidance and support. The

author expresses her warm respects and sincere thanks to other Assistant Professors, Dr.C.Shanmugapriya, Dr.Poonguzhali Gopinath, Dr.C.Mythili, Dr.V.Ananthan, Dr.S.Siva, Dr.B.SudhaPresanna, Institute of Biochemistry, Madras Medical College, for their valuable suggestions regarding the practical issues of research which is something beyond the textbooks.

The author expresses warm respects to the members of the Institutional Ethical committee for approving the study.

The author expresses her special thanks to Mr.K.Suresh and Mrs.Maragatham, Biochemists, Institute of Biochemistry, for their timely co-operation and assistance during the ELISA technique.

The author expresses her special thanks to her co-PGs Dr.P.Nirmaladevi, Dr.S.Anandhi and Dr.M.Divya, for their constructive criticism and unconditional support. The author expresses her thanks to all her colleagues in the institute, for their constant encouragement throughout the study period.

The author is grateful to the Statistician, Mr.K. Boopathi for his help in processing the data and statistical analysis.

The author is indebted to the patients and controls from whom blood samples were collected for conducting the study.

The author expresses her special thanks to all the DMLT students and the lab technicians of Rheumatology lab for their timely help and co-operation during sample collection and analysis.

Finally, the author expresses her special thanks to her family members for their moral support and encouragement.

CONTENTS

ABBREVIATIONS

1.	AI	- Autoimmune .
2.	ACPA	- Anti Citrullinated Protein Antibodies.
3.	AMPK	- 5’ Adenosine Monophosphate Activated Kinase.
4.	Bcl – 2	- Bcell lymphoma – 2
5.	Bcl – xl	- Bcell lymphoma extra large.
6.	CD	- Cluster differentiation
7.	CTLA4	- Cytotoxic T- Lymphocyte Associated protein-4.
8.	CaMKK2	- Calcium/Calmodium dependent protein kinase kinase 2.
9.	Fox01	- Fork head box protein 01
10.	FKHR- L1	- Fork head transcription factor like-1.
11.	GRB2	- Growth factor Receptor Bound protein 2.
12.	HLA	- Human Leukocyte Antigen
13.	IFN– γ	- Interferon– gamma.
14.	IRS-2	- Insulin Receptor Substrate -2
15.	JAK	- Janus Kinase.
16.	KDa	- Kilo Dalton
17.	MHC	- Major Histocompatibility Complex.
18.	MMP	- Matrix Metalloproteinases.
19.	MAPK/ERK	- Mitogen Activated Protein Kinase/ Extracellular Regulated Kinase.
20.	mTOR	- Mammalian Target Of Rapamycin.

21. NF-κB - Nuclear factor κB.

22. PGE2 - Prostaglandin E2.

23. PLC - Phospholipase C.

24. PKC - Protein Kinase C.

25. PI-3Kinase - Phosphoinositide-3 Kinase.

26. PPAR-γ - Peroxisome Proliferator Activated Receptor – gamma.

27. RA - Rheumatoid Arthritis.

28. RF - Rheu moratoid Factor.

29. SDF1 - Stromal cell Derived Factor-1

30. SOCS3 - Suppressors of cytokine signaling 3.

31. SP-1 - Specificity Protein 1.

32. STAT - Signal Transducers And Activators of Transcription.

33. TLR - Toll Like Receptors.

34. TH1 - T Helper lymphocyte 1.

35. TNF-α - Tumour Necrosis Factor alpha

36. TRAFI- C5 - Tumour necrosis factor – Receptor Associated Factor1/ Component 5.

37. UCP-1 - Uncoupling Protein 1.

SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS.

ABSTRACT

Aims and Objectives:

The fat stores in our body secrete many hormones and cytokines that not only regulate the energy balance but also regulate the inflammatory and immune responses. Among them leptin has been investigated widely as a proinflammatory cytokine in the pathogenesis of several inflammatory autoimmune diseases. Therefore this study was designed to measure the serum leptin levels in Rheumatoid Arthritis(RA) patients and to compare it with healthy controls and also to correlate serum leptin levels in Rheumatoid arthritis patients with their disease activity.

Methodology:

Sixty adult RA patients and 30 sex and age matched healthy controls were selected for this case control study. Serum leptin, C-reactive protein, lipid profile, uric acid, urea, creatinine and ESR were estimated in the study subjects. The disease activity in RA patients was assessed using the DAS 28(3) scoring system. Body mass index (BMI) was calculated for both RA patients and controls using Quetelet index. Estimation of serum leptin levels in RA patients and controls was done using DRG Leptin ELISA Kit.

Results:

RA patients showed statistically significant higher mean serum leptin level than healthy controls (48.78$\pm$ 32.00ng/mL versus 11.94$\pm$6.11ng/mL respectively, p=<0.001). However no significant correlation was observed in RA patients between

serum leptin level and disease activity (p= 0.240). But the serum leptin level showed a statistically significant positive correlation with body mass index (p= 0.001) and C-reactive protein (p= 0.001).

Conclusion:

As the serum leptin levels were significantly higher in the RA patients than in the controls it is understood that leptin plays a pivotal role in the pathogenesis of RA as a proinflammatory cytokine. However no correlation was observed between serum leptin levels and disease activity of the RA patients. But the serum leptin levels correlated well with body mass index indicating that the body fat stores can also decide upon the incidence of RA. The study also demonstrated a good correlation between serum leptin levels and C- reactive protein proving leptin as a inflammatory marker.

Keywords: Rheumatoid Arthritis, Serum Leptin , DAS28(3), BMI, C-reactive protein.

INTRODUCTION

Rheumatoid Arthritis is an autoimmune disease affecting 0.5-1% of general population.[1] Women are more often affected than men in the ratio of 3:1.[2] Recent studies have shown that the hormones secreted by the adipocytes like resistin, leptin and adiponectin act as new mediators of the inflammatory process.[3] Among all, leptin has been found to play a major role in the pathogenesis of Rheumatoid Arthritis.

Leptin is a 16KDa peptide synthesized by white adipose tissue(WAT). Leptin and its receptors Ob-R show structural and functional similarities with cytokines of IL-6 family and their receptors.[4] Though Leptin's major role is to regulate food intake and energy expenditure, few of the other important peripheral functions include regulation of endocrine function, reproduction and immunity. Proinflammatory mediators like IL-1, IL-6 etc also mediate leptin expression.. In both acute and chronic inflammation circulating leptin levels are found to be increased. Leptin modulates TH1/TH2 balance, regulating cytokines expression pattern. In synergy with other cytokines, leptin has been found to induce NOS type II activation in chondrocytes.[5]

Many clinical in vitro, in vivo animal and human studies suggests the involvement of leptin in the pathogenesis of Rheumatid Arthritis. Hence in this study we assess the role of leptin in Rheumatoid Arthritis and thereby aid in future prospective studies in the control of undesired leptin action in autoimmune inflammatory diseases.

REVIEW OF LITERATURE

RHEUMATOID ARTHRITIS

Rheumatoid Arthritis (RA) is a chronic systemic autoimmune inflammatory disorder characterised by symmetrical synovitis, polyarthritis, progressive joint damage, pain, fatigue and disability. It is due to complex interaction between genes and environment which causes break down of immune tolerance leading to symmetrical synovial inflammation.

EPIDEMIOLOGY:

A) PREVELANCE INCIDENCE OF RA

It is the most common inflammatory disorder affecting 0.5 – 1% of the general population in the world.[1] The prevalence of the disease is constant worldwide but few exceptions can be seen as in China where the incidence is as low as 0.3% and it is 5% in PIMA Indians of North America.[6] The onset of the disease is commonly seen in the fourth or fifth decade of life.[7] Most of the studies show a female : male ratio of 2:1 or 3:1.[2] Age specific prevalence rate have been found to increase with age and the sex difference has been found to decrease in older age group.

In the Indian adult population also it is the most common inflammatory disease with an incidence of approximately 0.75%.[8]

FACTORS ASSOCIATED WITH INCREASED RISK OF RA:

a) Host Factors:

i) Age:

The incidence of RA increases with advancing age. Old age is characterised by immune senescence. Due to immune senescence there is massive expansion of lymphocyte clone, erosion of leucocyte telomerase & corresponding contraction of naive T and B cell repertoires. Derangements are noted in pathways integral to antigen responsiveness and immune regulation. This leads to increased susceptibility to foreign pathogens, augmented reactivity to self-antigens and a repertoire of lymphocytes defective in tumour surveillance is generated. Therefore immune senescence in old age can be considered as a risk factor in the development of autoimmune diseases.[9]

ii) REPRODUCTIVE & ENDOCRINE FACTORS:

As more number of cases are seen among the female population and that too before menopause, a signifigant role by reproductive and hormonal factors have been suggested. Increased risk is seen among nulliparous women and those who have recently given birth. Higher incidence of rheumatoid arthritis is seen among women with history of preeclampsia, hyperemesis or gestational hypertension.

Breast feeding has been found to have reduced risk of developing rheumatoid arthritis.[10] Onset of menopause before the age of 45years has been found to be associated with increased risk of developing rheumatoid arthritis.[11]

Studies on the effect of oral contraceptive pills in the development of rheumatoid arthritis have been conflicting and some reports suggesting an inverse relationship with higher estrogen content.[12]

iii) BIRTH WEIGHT:

Birth weight more than 4Kg has been associated with increased risk of developing rheumatoid arthritis.[13] The exact pathophysiology is not known, but it is hypothesized that the pathology may be due to hypothalamo – pituitary axis dysfunction which is associated with both high birth weight and rheumatoid arthritis.

b) ENVIRONMENTAL FACTORS:

i) LIFESTYLE:

1) Cigarette Smoking:

Cigarette smoking, is the strongest known modifiable environmental risk factor[14]and has been found to be strongly associated with ACPA positive (Anti Citrullinated Protein Antibodies) and RF positive rheumatoid arthritis.[15] The risk increases with intensity (number of cigarettes per day) & duration of cigarette usage. Smokers who carry risk alleles in the Human Leukocyte Antigen Shared Epitope (HLA – SE) develop rheumatoid arthritis and this suggests the role of gene– environment interaction in smokers. Smoking increases the proportion of citrulline +ve cells in the lungs, which provides systemic antigen exposure that can contribute to Anti-CP antibody production and risk of developing rheumatoid arthritis later.[16]Other genes that have been

implicated to increase risk of developing RA among smokers include genes coding for enzymes that detoxify carcinogens namely

- Glutathione S transferase genes
- Hemeoxygenase gene 1 (*HMOX1*)
- N – Acetyl transferase 2 (*NAT2*)

2) Alcohol:

Studies have shown that alcohol consumption reduces the risk of developing RA, especially ACPA+ve RA.[17]

Obesity:

Obese individuals with body mass index of more than 30 have been found to be associated with increased incidence of RA.[18]

NUTRITION:

Influence of diet on the risk of developing RA is variable. Some studies suggested a high protein diet and red meat intake is associated with increased risk of inflammatory arthropathy.[19]Consumption of olive oil and fish oil has been reported to protect against the risk of developing rheumatoid arthritis.

- Vitamin D being a modulator of immune response, its deficiency is found to be associated with increased risk of developing RA.
- Some studies have suggested a link between RA & deficiency of copper and selenium. Vitamin C rich diet reduces the risk of inflammatory polyarthritis.

ii) MEDICATIONS:

Use of statins by individuals with hyperlipidemia reduces the risk of developing RA as statins have modest anti-inflammatory effects.[20]

INFECTIOUS AGENTS:

There is no epidemiological evidence for an infectious cause for rheumatoid arthritis. In some patients after diagnosing rheumatoid arthritis increased titres of antibodies to Ebstein Barr virus have been found. [21]In females with rheumatoid arthritis antibodies to human parvo virus was found to be increased.[22]

iii) SOCIOECONOMIC STATUS AND OCCUPATION:

In recent studies an inverse relationship has been found between socioeconomic status and risk of rheumatoid arthritis.

Exposure to silica dust and mineral oil is associated with increased risk of rheumatoid arthritis.[23]

iv) URBAN AND INDUSTRIAL ENVIRONMENT:

A general association has been found between rheumatoid arthritis and urban industrialised environment. Traffic pollution exposure is associated with increased risk for rheumatoid arthritis.[24]

ETIOLOGY:

The etiology of rheumatoid arthritis remains unknown, but a variety of studies have suggested an interaction between environmental and genetic factors responsible for development of rheumatoid arthritis.

Family studies have indicated a genetic predisposition. Severe rheumatoid arthritis has been seen in the 1st degree relatives of individuals having disease associated with presence of autoantibodies, RF; and it is approximately 4 times the expected rate. It has also been observed that approximately 10% of rheumatoid arthritis patients will have an affected 1st degree relative. Moreover monozygotic twins are 4 times concordant for rheumatoid arthritis than the dizygotic twins,who have a similar risk as non twin sibling. Only 15 -20% of monozygotic twins are concordant for rheumatoid arthritis implying that factors other than genetics play an important role in the etiopathogenesis of RA.

In immunogenetics, the most influential genetic risk factor has been the class II MHC haplotype of an individual. Protein Tyrosine Phosphatase 22 (PTPN22) and Peptidyl Arginine Deiminase 4 (PADI4) increases the risk of rheumatoid arthritis in some ethnic and racial groups. So far 35 genes involved with immune function have been implicated. Combination of HLA - DR, PTPN22 and TRAF1-C5 interact with one another and cause a 45 fold increase in the risk.[25] This combination is seen in less than 1% of the individuals with rheumatoid arthritis.

i) ROLE OF HLA-DR:

The increased susceptibility and severity of rheumatoid arthritis is associated with the structure of class II MHC molecules present on the antigen presenting cells and it accounts for about 40% of genetic influence. 70% of rheumatoid arthritis patients have HLA-DR4 compared to 30% of the controls, and so the relative risk of developing rheumatoid arthritis in HLA-DR4 individuals is approximately 4 to 5.

The third hypervariable region of DRβ chains from aminoacids 70 to 74 is associated with increased susceptibility to rheumatoid arthritis. The sequence of the epitope found in DR4, DR14 and some other DR1β chain is glutamine – leucine – arginine – alanine –alanine (QKRAA). The "susceptible epitope" (SE) on DR4β chain that has great association with rheumatoid arthritis are DRB*0401, DRB*0404, DRB*0101and DRB*1402.[26] Some HLA genes like DRB*1301 have DERAA sequence that has decreased susceptibility to rheumatoid arthritis.[27]

Patients who do not fit into this paradigm exhibit microchimerism.[28] The susceptible epitope (QKRAA) expressed by the maternal cells can persist throughout adulthood and these non inherited maternal antigens (NIMA) confers increased risk.

Molecular mimicry is also observed in some situations where the susceptible epitope (QKRAA) serve as an autoantigen. Certain proteins such as gp110 from Ebstein Barr virus also share this sequence.

ii) ADDITIONAL POLYMORPHISM: CYTOKINES, CITRULINATING ENZYMES, PTPN22 AND OTHERS:

Some non MHC genes have also been evaluated for genetic influence on rheumatoid arthritis. Single nucleotide polymorphism(SNP) has been studied extensively in rheumatoid arthritis. Table below shows some of the SNPs and microsatellites associated with rheumatoid arthritis.

TABLE1: Genetic associations in RA.

Gene	Odd's Ratio For Risk Alleles	Comment
HLA-DR	4-5 fold	
PTPN22	=2fold	
PADI4	=2fold	
TRAFI-C5	>1.2 fold;<2fold	Not in Asian populations
STAT4	>1.2 fold;<2fold	Primarily in Asian populations
TNFAIP3	>1.2 fold;<2fold	
IL2/21	>1.2 fold;<2fold	

Courtesy KELLY'S Textbook of Rheumatology, 9th edition, pg.1062.

Cytokines also play an important role in the pathogenesis of rheumatoid arthritis, among which Tissue necrosis factor(TNF) plays a major role. It's genes are located on chromosome 6.[29]

The noncytokine and non MHC genetic linkages involved in rheumatoid arthritis are :

- Peptidyl arginase deiminase (PADI)
- Protein Tyrosine phosphatase 22 (PTPN22)

Four isoforms of PADI genes are identified, PADI1 to PADI4. PADI4 gene SNPs are noted in Japanese population and they have a twofold increased risk for rheumatoid arthritis.[30]

PTPN22 gene SNPs have also been found to have a twofold increased risk in the development of rheumatoid arthritis. PTPN22, a phosphatase regulates the phosphorylation of various kinases required for T cell activation.[31]

Genes associated with rheumatoid arthritis, regulating adaptive immune responses in T cells include:

- Protein Tyrosine Phosphatase – 22 (PTPN22)
- Cytotoxic T – lymphocyte associated protein (CTLA)

Other genes implicated in rheumatoid arthritis and associated with B – cell and /or antigen presenting cells are

- B and T – lymphocyte attenuator (BTLA)
- Fc receptors
- CD40

Signal transduction pathways regulating the immune function like TRAFI – 5 and STAT 4 have also shown exhibit polymorphism. [32]

iii) INTERACTION BETWEEN GENES AND ENVIRONMENT:

There are many environmental factors contributing to rheumatoid arthritis but among them smoking has been considered as a major environmental risk factor for seropositive rheumatoid arthritis. In smokers there is activation of innate immunity and PADI in the airway and this has led to the citrullination of proteins which are found in their bronchoalveolar lavage. This then stimulate the generation of ACPAs in susceptible individuals leading to development of rheumatoid arthritis. Smokers with two copies of "SE" have a 40 fold increased risk of developing rheumatoid arthritis due to the increased ability of the HLA-DR molecules containing the "SE" to bind some citrullinated proteins. Also studies show that the risk declines only slowly after cessation of smoking and may take more than a decade to reach that of non smokers.

Alcohol consumption has been found to decrease the risk, while exposure to inhaled irritants like silica dust increases the risk of developing rheumatoid arthritis. Oral contraceptive pills has been observed for its protection from rheumatoid arthritis, due to the changes in the hormonal balance.

Therefore there is complexity in understanding the environment and human behaviour on disease susceptibility.

iv) GENDER:

Rheumatoid arthritis is one among the many autoimmune diseases that predominate in women. The ratio between male and female patients is 1:2 to 1:3. This has been found to be due to estrogens and some studies have supported the concept that the immune function is modulated by estrogens. Fibroblasts like synoviocytes(FLS) express estrogen receptors increasing the production of metalloproteinases. Moreover estrogen causes enhanced production of tissue necrosis factor (TNF) by macrophage cell lines.

The last trimester of pregnancy in rheumatoid arthritis patients is often associated with remission of disease. This has been thought to be due to alterations in cell mediated immunity, expression of suppressive cytokines like IL-10 or production of α-fetoprotein.[33]

V) EPIGENETICS:

Epigenetics describes the phenotypic or genome expression properties caused by mechanism other than changes in the underlying DNA. In rheumatoid arthritis most information on epigenetics comes from studies on rheumatoid arthritis synovium or cultured synoviocytes. In rheumatoid arthritis synovium low levels of DNA methylase(Dnmtl) is expressed.[34] Ingestion of methyl donors like folate or exposure to methyl donors in utero can alter methylation of DNA and adaptive immune function, thereby affects the susceptibility to autoimmune disease.

PATHOGENESIS:

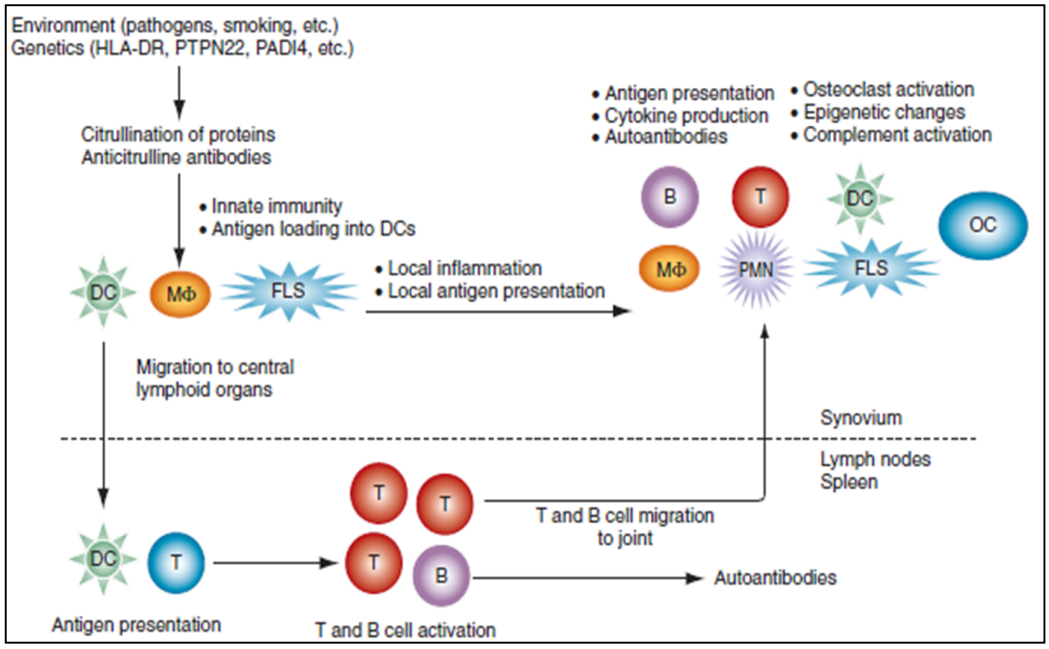


Figure1: Disease mechanism in RA.

Courtesy KELLEY'S Textbook of Rheumatology. 9th edition, pg.1060.

A single specific " rheumatoid arthritis pathogen" is not established but viruses, retroviruses, bacteria and mycoplasma have been associated with rheumatoid arthritis. Due to repeated inflammatory stress through specialised receptors that respond to common molecules produced by the pathogens, in a genetically susceptible individual might lead to breakdown of tolerance and subsequent autoimmunity.

I) INFECTIOUS AGENTS: DIRECT INFECTION AND INNATE IMMUNITY RESPONSES:

Infectious agents can initiate immune response by various mechanisms.

TABLE2: Possible infectious causes of RA.

Infectious Agents	Potential Pathogenic Mechanisms
Mycoplasma	Direct Synovial Infection; Superantigens.
Parvovirus B19	Direct Synovial Infection.
Retroviruses	Direct Synovial Infection.
Enteric bacteria	Molecular mimicry (QKRAA,e.g., In Bacterial Heat Shock proteins)
Mycobacterium	Molecular mimicry (Proteoglycans,QKRAA), Immunostimulatory DNA (Toll-like receptor 9 activation.)
Epstein-Barr virus	Molecular mimicry (QKRAA in gp110)
Bacterial cell walls	Toll-like receptor 2 activation.

Courtesy KELLEY'S Textbook of Rheumatology, 9th edition, pg 1065.

Toll like receptors, expressed by the sentinel cells of the host provide first line of defence. These receptors could recognize the preserved structures of the bacteria and other infectious agents leading to release of inflammatory mediators, activating the antigen presenting cells and enhancing adaptive immune responses.

In human beings at least 11 Toll-like receptors are identified. TLR2 binds peptidoglycans, TLR3 binds double stranded RNA (dsRNA), TLR4 binds lipopolysaccharide and TLR9 binds bacterial DNA containing CpG motifs. Rheumatoid synovial tissue expresses many of these pattern recognition receptors. "Exogenous" TLR ligands like bacterial peptidaglycan and DNA, as well as "endogenous" ligands like heatshock protein, hyaluronan and fibrinogen are seen in arthritic joints.[35]

Inflammasome is a novel structure that regulates the innate immunity through the second mechanism. This complex consists of several proteins that recognises the “danger signals” and pathogens like muramyl peptides and uric acid. Cryopyrin (NALP3) is one such complex, that is linked to caspase 1(IL-1convertase). So when the inflammasome gets engaged caspase 1 is activated and IL-1 is produced.

i) BACTERIA, MYCOBACTERIA, MYCOPLASMA AND THEIR COMPONENTS:

Most rheumatoid arthritis synovium contains bacterial DNA sequence such as that of Acinetobacter and Bacillus spp. The “synovium” functions as an adjunct to the reticuloendothelial system in arthritis and allows the local macrophages in accumulating the circulating bacterial products. TLRs are expressed by the antigen presenting cells containing these products and these produce proinflammatory cytokines like TNF. Superantigens of mycoplasma, such that of Mycoplasma arthridis can directly cause induction of T-cell independent cytokine production by macrophages, thus exacerbating or triggering arthritis. Higher prevalence of antimycoplasma pneumonia IgG antibodies is seen in rheumatoid arthritis patients than in controls.[36]

ii) EBSTEIN- BARR VIRUS, DNA J PROTEINS AND MOLECULAR MIMICRY:

Ebstein Barr Virus is an activator of polyclonal B lymphocyte and increases the production of Rheumatoid Factor. Moreover there is defective

suppression of EBV induced proliferation of B cells by rheumatoid macrophages and T cells. This has been attributed to the homology between the "susceptibility cassette" in HLA-DR proteins and the EBV glycoprotein gp110. Like DRB*401, QKRAA motif is seen in gp110 protein and serological evidence of antibodies against this epitope is observed in patients with previous history of EBV infection.

Therefore the T cell recognition of EBV epitope in some patients with "SE" can cause an immune response directed against the synovial cells through "molecular mimicry".

Like gp110 there are many other xenoproteins exhibiting the same QKRAA motif and among them Escherichia coli heat shock protein dnaJ is important. Rheumatoid arthritis T cells, especially the synovial fluid T cells show increased proliferative response to gp110 thus supporting the molecular mimicry link existing between a variety of QKRAA containing proteins and rheumatoid arthritis.[37]

iii) PARVOVIRUS:

Parvovirus B19 DNA has been detected in 75% of rheumatoid arthritis synovium. B19 protein VP-1 has been detected in patients with rheumatoid arthritis. The mechanism involved in B19 induced synovitis has been related to alteration in FLS function.

OTHER VIRUSES:

Studies of synovial tissues have demonstrated DNAs of many other viruses like rubella virus, cytomegalovirus and herpes simplex virus. It has been observed that one or more of these infections could trigger the disease in the genetically susceptible host.

II) AUTOIMMUNITY:

There is evidence of autoimmunity present in patients with rheumatoid arthritis many years before the onset of clinical symptoms. Autoantibodies commonly associated with rheumatoid arthritis include RF and anticitrullinated protein antibodies(ACPAs).

Autoantibodies in rheumatoid arthritis recognizes joint antigens like Type II collagen or systemic antigens like glucose phosphate isomerase leading to synovial inflammation through several mechanisms.

TABLE 3 Autoantigens in RA

Cartilage Antigens	Other Antigens
Type II Collagen	Immunoglobulin G
gp 39	Citrullinated proteins
Proteoglycans	Glucose-6-phosphoisomerase
Aggrecan	HLA-DR (QKRAA)
Cartilage link protein	Heat shock proteins

Courtesy KELLEY'S Textbook of Rheumatology, 9th edition, pg 1069.

i) RHEUMATOID FACTOR (RF):

The first direct evidence of autoimmunity in rheumatoid arthritis was established when RF, an autoantibody that can bind Fc portion of IgG was identified and studied. The RF and other autoantibodies are identified in patients with rheumatoid arthritis many years before the onset of disease.[38] Some patients are initially "seronegative" and they become "seropositive" within the first year of disease activity.

Patients with RF positivity in blood exhibit more severe form of disease including severe cardiovascular complications.[39]It has been demonstrated that RF can fix and activate complement by classic pathway and also cause local complement production and consumption in rheumatoid joint. Rheumatoid synovial tissue produces large quantities of IgG RF forming complexes through self association. Rheumatoid arthritis synovial tissue and surface layers of cartilage express RF containing the immune complexes.

Seventy five percent of rheumatoid arthritis patients are seropositive by usual standard tests but it can be as high as 90% when IgM RF is assayed using enzyme linked immunoassay. First degree relatives of seropositive patients are frequently found to be RF+ve suggesting a genetic predisposition. IgG and IgM RFs are the most abundant in rheumatoid arthritis patients but in patients with extraarticular manifestations IgE RF has also been demonstrated.

ii) Anticitrullinated Protein Antibodies (ACPAs):

Immunoglobulins binding to citrullinated proteins are produced by rheumatoid arthritis patients. It is one of the most striking features recently observed and it has significant prognostic implication. Antibodies directed against keratin were detected in serum of rheumatoid arthritis patients and the primary target antigen was Filaggrin, a filament aggregating protein. These antibodies bind to epitopes on filaggrin containing citrulline , which is derived by post translation modification of arginine by PADI. Four isoforms of PADI are observed in humans and among them PADI2 and PADI4 are abundant in synovium.[40] It has been studied that certain SNPs in Asian population are associated with rheumatoid arthritis.

CPs of the smokers' lungs provide a systemic antigen exposure that can lead to production of anti CP antibodies predisposing to rheumatoid arthritis. Rheumatoid synovium especially tissues with lymphoid aggregates produce ACPAs.

Serum of 80-90% of the patients demonstrate ACPAs. The specificity of ACPA is near 90% and it is more specific than RF. Like RF, ACPAs are detected in patients with rheumatoid arthritis long before the onset of clinical arthritis. A genetic predisposition has been confirmed in Native American population, where 20% of unaffected 1st degree relatives have ACPAs in their serum and it is detected in more than 10% of distant relatives.

Presence of ACPAs predict an aggressive disease marked by destruction of bone and cartilage. Anti CP positivity is observed as an independent risk factor for coronary artery disease.

ACPAs activate both classical and alternative complement pathways.[41] In addition Ig E ACPAs in patients with rheumatoid arthritis sensitizes degranulation of basophils and mast cells. This enhances the vascular permeability and infiltration of the synovium by inflammatory cells.

Type II collagen which is citrullinated is found to be more immunogenic than the native protein and this is attributed to increased affinity in the binding groove of HLA-DR proteins that contain the "SE".

iii) Autoimmunity to cartilage specific antigens:

As synovial tissue inflammation is a hallmark of rheumatoid arthritis some joint specific antigens play a pathogenic role. Though there are a number of potential antigens there is no single antigen as "rheumatoid" antigen.

a) Type II Collagen:

For initiation of collagen induced arthritis T cells are required and a major arthrogenic and immunogenic epitope is found to reside in a" restricted area" of the type II collagen chains. Type II collagen antibodies amplify the inflammatory process. Antibodies to denatured bovine type II collagen are demonstrated in sera of rheumatoid arthritis patients which is significantly higher than that of control sera.[42] When the purified anticollagen antibodies

from the sera of the patients bind to the cartilage, they activate the complements and generate C5a.

b) Glycoprotein39(gp 39):

Another cartilage component that has been implicated as a potential autoantigen in rheumatoid arthritis is cartilage glycoprotein 39 (gp39). This gp39 peptides bind to HLA-DR*0401 of rheumatoid arthritis patients and stimulate the proliferation of T cells. Only in a small percentage of patients antibodies to gp39 are detected but it happens to be specific for rheumatoid arthritis.

c) Other cartilage specific antigens:

Proteoglycans, aggrecan and cartilage-link protein are few other examples of potential cartilage autoantigens.

iv) Autoimmunity to non articular antigens:

Antigens beyond the joint also can cause autoimmune responses in RA.

a) Glucose -6-Phosphoisomerase:

Glucose- 6- Phosphate isomerase(GPI) adheres to the cartilage surface and allows local antibody binding and fixation of the complement. Mast cells, the alternate complement pathway and the Fc receptors are required for initiation of synovial inflammation in this GPI induced arthritis, moreover IL-1 is more important in this model.[43]

Heterogenous Nuclear Ribonucleoprotein A2:

About one third of rheumatoid arthritis patients exhibit antibodies directed against heterogenous nuclear ribonuclear protein A2(hnRNP-A2), otherwise called RA33. Less destructive disease has been observed in this RA33 positive patients. Though it is not sensitive or specific for rheumatoid arthritis, this along with RF and ACPAs can be used to predict the risk of erosive disease in patients with early synovitis.[44]

Heavy chain binding protein:

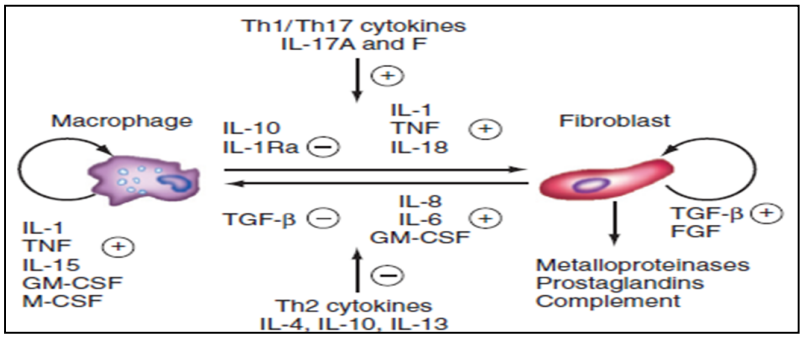
Autoantibodies binding to stress protein immunoglobin heavy chain binding protein (BiP) have been identified. Anti BiP antibodies is present in 60% of patients and the specificity is more than 90%.

b) Heat shock proteins:

Heat shock proteins are produced by cells of all species in response to stress. Synovitis and joint destruction are caused by autoantibodies of heat shock proteins.

ROLE OF T-CELL CYTOKINES:

Figure 2 Cytokine network in RA



Courtesy KELLEY'S Textbook of Rheumatology, 9th edition, pg 1093.

Several subsets of T cells are involved in the etiology of rheumatoid arthritis. In rheumatoid arthritis factors produced by macrophages and fibroblast dominate and that of T cells are surprisingly low. T cell cytokines like IFN-γ and IL-17 are produced by Th1 or Th17 cells. Regulatory T cell function is found to be low in rheumatoid arthritis synovium.

i) T Helper Type I Cell Cytokines:

Th1 phenotype produces cytokines like IL-2 and IFN-γ and expresses chemokine receptors CXCR3 and CCR5. IFN-γ is a potent inducer of HLA-DR antigens. IFN-γ causes alteration in the balance of extracellular matrix synthesis and degradation by decreasing synthesis of collagen and inhibiting the production of matrix metalloproteinase by cytokine stimulated FLS.[45]

ii) T Helper Type 2 Cell Cytokines:

IL-4, LT-α, IL-12, IL-13 and IFN-γ are the cytokines produced by Th 2 cells and these are found in the rheumatoid synovium. IL-12 is a cytokine that causes T cell maturation towards the Th1 phenotype.[46]

iii) T Helper Type 17 cytokines:

Th 17 cells produce the proinflammatory cytokine IL-17 which exists in six forms (IL-17A through IL-17F). IL-17A and IL-17F mimic IL-1 and TNF in many of the activities related to FLS which includes collagenase induction and cytokine production. IL-17A is detected in modest amounts in synovial effusions. [47]

iv) Regulatory T cells (Tregs):

The CD4+, CD25+ and CD127+ cells are deficient in rheumatoid arthritis patients. Autoimmunity is attributed to the deficiency of this subset, which produces TGF-β and IL-10 and regulates the T cells through cell contact.

T Helper Cell Imbalance In Rheumatoid Arthritis:

In rheumatoid arthritis the Th1 phenotype and Th17 are demonstrated in relative abundance to the Th2 phenotype. The rheumatoid synovium exhibit Th1- like delayed type hypersensitivity reaction and /or a Th 17 autoimmune environment.

ROLE OF MACROPHAGES AND FIBROBLAST CYTOKINES:

Cytokines of macrophage and fibroblast are found abundantly in rheumatoid synovium. Proinflammatory cytokines include IL-1, TNF, IL-6, IL-15, IL-18, GM-CSF and IL-33. Chemokines produced by the macrophage and fibroblast drive inflammatory cells into the joint.

Although anti-inflammatory cytokines like IL-Ra and IL-10 are produced by rheumatoid synovium , they are insufficient to suppress the function and production of proinflammatory cytokines.

I) Proinflammatory Macrophage And Fibroblast Cytokines:

A) Interleukin -1 Family:

The IL-1 family includes a variety of polypeptides with a wide range of biological activity and they include IL-1α, IL-1β, IL-18, IL—33 and IL-Ra, a natural inhibitor of IL-1.

i) **IL-1:** IL-1 is secreted abundantly by synovial macrophages, especially IL-1β.[48] The IL-1activate B synoviocytes to proliferate and secrete a variety of mediators like IL-6, IL-8, GM-CSF and enhances the production of collagenase and prostaglandin.

ii) **IL-18:** IL-18, in the presence of IL-12 has the ability to bias the immune response towards the Th1 phenotype. When IL-18 is inhibited in collagen induced arthritis, there is attenuation of the disease.[49] Moreover IL-18 induces GM-CSF, expression of TNF and nitric oxide production by synovial macrophages.

iii) **IL-33:** It is a novel cytokine signalling through ST2 surface recptors. It triggers the mast cells to release TNF-α and IL-17A in rheumatoid synovium.

B) Tumour Necrosis Factor:

The TNF superfamily belongs to an extended group of related genes that has been found to play a major role in immune responses, inflammation, cell survival and apoptosis. TNF plays a key role as a proinflammatory cytokine in rheumatoid arthritis. It is detected in both rheumatoid serum and synovial fluid. TNF and IL-1 have many similar functions like enhancing cytokine production, expression of adhesion molecules, proliferation and MMP production. They have been found to share many functions and signal transduction pathways but the surface receptors and the intracellular signalling pathways they use are different.

TNF functions include

- Stimulation of PGE2 and collagenase production by human synovial cells.
- Induction of bone resorption and inhibition of bone formation in vitro.
- Inhibition of proteoglycan resorption and its biosynthesis in cartilage explants.

TNF blockade has been proved to be an effective anti inflammatory agent and also it has been observed to decrease significantly the destruction of extracellular matrix which has been measured by radiographic progression.[50]

C) IL-6 Family:

IL-6, a complex cytokine is produced by many types of cells like monocytes, T cells and FLS. Its functions include stimulation of B cells to produce immunoglobulins, differentiation of cytotoxic T cells and regulation of acute phase reactants like C-reactive protein.

D) IL-12 Family:

It is a heterodimeric cytokine containing p35 and p40 as its subunits and they play a key role in T cells differentiation and inflammation. The protein is produced by the macrophages in the rheumatoid joint and also by other antigen presenting cells like the dendritic cells.

E) IL-15:

IL-15 resembles IL-2 and regulates T cell chemotaxis and proliferation, production of immunoglobins by B cells and generation of natural killer (NK) cells. Macrophages are the primary source of IL-15in RA.

F) IL-32:

A novel cytokine, it activates NFκB pathway and induces the production of TNF, IL-1, IL-6 and IL-8. It has been demonstrated in the synovial lining macrophage- like cells.[51]

G) Granulocyte-Macrophage Colony Stimulating Factor(GM-CSF):

GM-CSF is a potent macrophage activator and causes induction of HLA-DR expression, IL-1 secretion, priming for increased release of TNF and PGE_2. It also regulates neutrophil function by antibody dependent cytotoxicity, phagocytosis, chemotaxis and the production of oxygen radical. RA synovial tissue cells produce GM-CSF which can be detected in RA synovial fluid.[52]

H) Chemokine Families:

Chemokines belongs to a family of chemoattractant peptides, which with the help of adhesion molecules, attract the cells into the site of inflammation. The families include CC, CXC and CX3C which are classified according to the position of the characteristic cysteine residues.

IL-8, a CXC chemokine, is a potent neutrophil chemoattractant. This chemokine along with immune complexes and chemotactic peptides such as

C5a, lead to large influx of PMNs into the joint. IL-8 protein is demonstrated along the sublining perivascular macrophages.[53]

CXC family of chemokines include CXCL16 and Epithelial Neutrophil Activating Peptide-78 (ENA-78). CXCL16 binds to CXCR6 on T cells and contribute to the influx of lymphocytes into the synovium. 40% of the chemotactic activity of neutrophils in the RA synovial fluid is performed by ENA-78.[54]

CXCL13 is expressed by the follicular dendritic cells of germinal centres found in the RA synovial tissues. They cause B cell migration in the RA joints.[55]

SDF-1 is another major chemokine which is expressed by synoviocytes and endothelial cells of RA joints. They play a major role as T cell chemoattractant in the synovium through its receptor CXCR4. CXCR4 is highly specific for SDF-1and it is expressed by memory $CD4^+$ lymphocytes.

I) Platelet Derived Growth Factor (PDGF):

PDGF, a potent growth factor, is both chemoattractant and mitogenic for fibroblasts inducing collagenase expression. The isoforms of this molecule include PDGFA through PDGFD and all are detected in the RA synovial membranes.

J) Fibroblast Growth Factor(FGF):

FGFs belong to a group of peptide growth factors that stimulates angiogenesis. FGFs are present in RA synovial fluid and are expressed by synovial cells.

SUPPRESSIVE CYTOKINES AND CYTOKINE ANTAGONISTS:

A number of suppressive and anti-inflammatory factors try to re-establish homeostasis by suppressing the proinflammatory cytokine network in RA. These include IL-1RA, IL-10 and Transforming Growth Factor- β. It has been found that reduced production of these suppressive cytokines could lead to the perpetuation of the synovitis.[56]

CLINICAL FEATURES:

In more than 2/3 rd of the patients there is an insidious onset with fatigue, generalized weakness and vague musculoskeletal symptoms. This persists for weeks or months before the specific symptoms develop. In approximately 10% of individuals there is an acute onset with rapid development of polyarthritis.

There is pain, swelling and tenderness in the joints of hands, wrist, knees and feet. RA commonly causes symmetric arthritis usually affecting the proximal interphalangeal joints and metacarpophalangeal joints. Synovitis of wrist and knee joints are common features of RA. Axial involvement is usually limited to upper cervical spine.

Extra articular manifestations include rheumatoid nodules, rheumatoid vasculitis, interstitial fibrosis, pneumonitis, pericarditis, Sjogren's syndrome, Felty's syndrome and osteoporosis.[57]

BIOMARKERS IN RHEUMATOID ARTHRITIS:

Acute phase reactants: ESR, CRP, IL-6.

Autoantibodies: IgM RF, IgA RF, ACPA.

Synovial vascularity: VEGF.

Cartilage metabolites: MMP1 & 3, COMP – Cartilage oligomeric protein, C- terminal collagenII.

Bone metabolites: Pyridonoline cross links, Carboxy terminal collagen I telopeptides.

C- reactive protein(CRP):

CRP indicates disease activity in many inflammatory conditions. It is also used as a marker in RA. It was discovered by Tillet and Francis in the year 1930, in acutely ill patients as a protein that binds to cellwall C- polysaccharide of streptococcus pneumonia.

It has a molecular weight of 23,028 Da and is made up of 5 identical polypeptide subunits. It has a disc shaped structure with radial symmetry. Its half life is 18 to 24 hours. It is related to serum Amyloid P and pentraxin-3. It can provide nonspecific host defence and can activate classical complement pathway.

A serum concentration of 5-10mg/L suggest an inflammatory process and it is also proportionate to tissue damage.[58]

LEPTIN

Leptin, meaning "Leptos"(Greek word – thin), is a16kDa peptide containing 167aminoacid.[59] It is produced by leptin gene that was originally discovered in ob/ob mice through positional cloning.[60] It has been found to be structurally and functionally related to the type I cytokine superfamily. Though it plays a major role in food intake and energy homeostasis, it also has other functions as in hypothalamic neuroendocrine regulation, insulin resistance, haematopoiesis, reproduction, inflammatory and immune response.[61]

Leptin Gene:

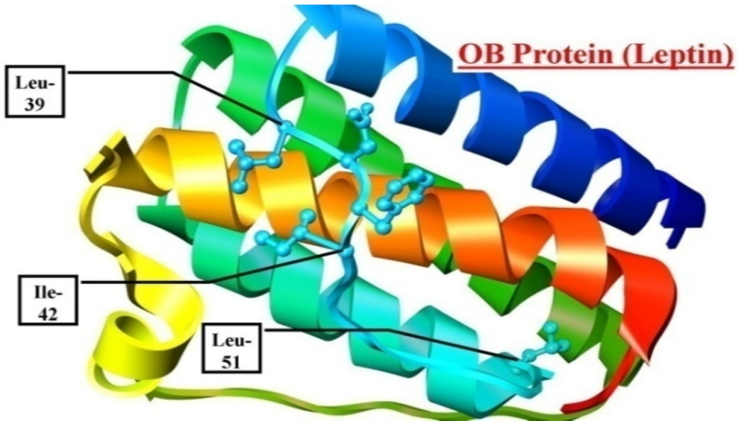


Figure 3 : Adapted from thenaturalhistorian.com

The ob protein, leptin was discovered in 1994 at Rockfeller University by a team led by Jeffrey Friedman.[62]It encodes mRNA transcript of 4.5 kilobase. The human ob gene has been localized on chromosome 7q31.3.[63] There are 3 exons and 2 introns encompassing 650 kb. The exons 2 and 3

contain the coding region of the ob protein. Initial studies have identified several regulatory elements within the promoter region of ob gene which includes cyclic AMP and glucocorticoid response elements, C/EBPα(CCAAT/enhancer binding protein), SP-1 and PPARγ binding sites. [64] It has been observed that 84% of human leptin is identical to that of mouse and 83% to that of rat leptin. It has been discovered that there is an intrachain disulphide bond that plays a significant role in the biological activity of leptin.[65]

Structural analysis of the molecule has suggested leptin as a globular protein whose tertiary structure resembles that of the haematopoietic cytokines like interleukins and GMCSF.[66]Leptin is produced mainly by white adipose tissue(WAT). A strong correlation has been found between leptin mRNA and adipose tissue protein levels and circulating levels of leptin. Leptin secreted by adipocytes is directly proportional to the amount of fat present in an individual[67]Moreover it has been observed that larger adipocytes contain more ob mRNA than the smaller adipocytes isolated from the same individual.

Very few human ob mutations have been identified so far. Montague et al first reported two children of consanguineous Pakistani family having ob gene mutation due to frameshift mutation.[68]Another three members of theTurkish family were identified by Strobel et al as having ob mutation due to missense mutation.[69] Individuals having ob gene defect exhibit hyperphagia, hypothalamic hypogonadism and morbid obesity. Patients have been found to

have increased risk of mortality due to infections as there is immune system dysfunction.[70]

Leptin expression and regulation:

The energy stores of fat in an individual decides the leptin expression. It has been observed that adipose ob mRNA and serum levels of leptin are increased in obese humans and in other mammals also.[71]Leptin levels increase in rodents within hours after a meal and in humans only after several days of overfeeding and it decreases in both the species within hours after initiation of fasting**.** Therefore the energy stores and energy balance are maintained by leptin.

Table 4 Regulation of Leptin expressio

Site	Increase	Decrease
Adipose tissue	Overfeeding Obesity (except ob mutation) Insulin Glucocorticoids Acute infection Cytokines (TNF-α, IL-1, LPS)	Fasting Testosterone Beta-adrenergic agonists Thiazolidinediones (in vitro) ? Thyroid hormone Cold exposure
Placenta	Insulin Glucocorticoids Hypoxia	Smoking Low birth weight
Skeletal muscle (rat)	Glucosamine Glucose Lipids	
Stomach fundus (rat)		Feeding Cholecystokinin

Courtesy Leptin Rexford S.Ahima pg 416

Leptin expression by nutrition is partly regulated by insulin. In the feeding cycle when the insulin levels peak there is increased leptin expression**.**[72]**.** Leptin is expressed in response to cortisol also. An inverse correlation has been observed between the pulsatile secretion of the leptin and

cortisol and ACTH.[73]Higher levels of leptin are seen in the evening and early morning.[73]

There is a prepubertal spurt of leptin expression and this has been related to the gonadal axis maturation.[74]Females have been found to have higher leptin levels and this has been attributed to higher fat content and low testosterone levels.[75]Leptin synthesis is inhibited by testosterone but not by ovarian steroid hormones.[76]

In euthyroid patients there is correlation between leptin, TSH and adipose tissue. But in hypothyroidism and in hyperthyroidism there are conflicting reports.[77]

Infections, endotoxins and cytokines like IL-1, TNF, LIF stimulate synthesis of leptin.[78.79] The secretion of leptin is decreased by cold exposure and catecholamines probably through β-adrenergic receptors.[80,81]

Leptin is also secreted by extra adipose tissues like gastric fundic mucosa, skeletal muscle, placenta and mammary epithelia.[82,83]There is decreased synthesis of leptin in gastric fundus and increase in serum leptin on administrarion of cholecystokinin or gastrin and on feeding. [83]Glucocorticoids, insulin and hypoxia stimulate leptin expression in the placenta.[84]Mammary epithelia also synthesize leptin which is secreted in the colostrums and absorbed by neonates.[85]

Increased expression of leptin in adipose tissue and denovo synthesis of leptin in the skeletal muscles of rats are observed on glucosamine infusion.[86] Administration of inflammatory stimuli like lipopolysaccharide (LPS) or turpentine increased leptin expression in adipose tissue and the circulating levels of leptin in hamsters. In sepsis a significant elevation in leptin levels have been demonstrated.

DB GENE:

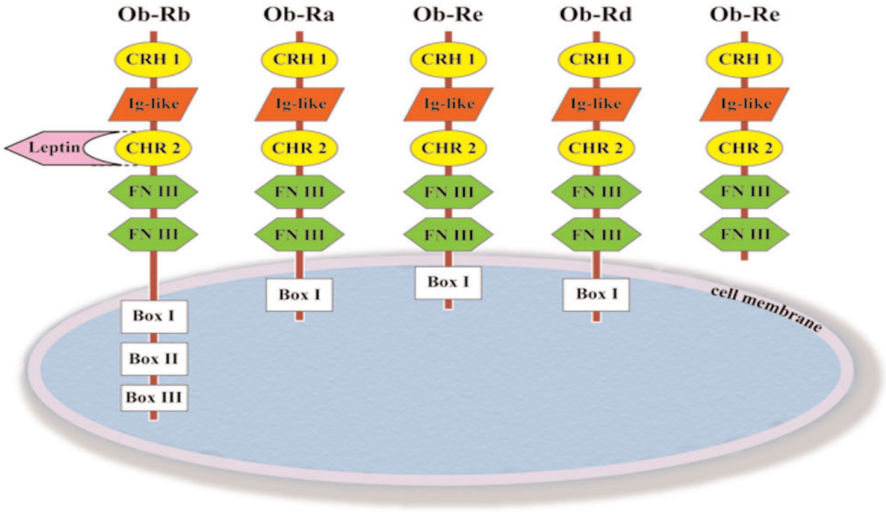


Figure 4 : Courtesy Eiva Bernotiene et al,2006.

Leptin receptor (Ob-R) was first isolated by Tartaglia et al from the choroid plexus of the mouse.[87]It is a protein receptor that is encoded by the diabetes (db) gene. The Ob-R mRNA encodes six leptin receptor isoforms.[88]They include the long form Ob-Rb, the short form Ob-Ra,c,d,f and the soluble form Ob-Re. It has been observed that the leptin receptor belongs to the family of class I cytokine receptors which include Interleukin-6(IL-6), Granulocyte colony stimulating factor(GCSF), leukemia inhibitory factor(LIF)

and glycoprotein 130(gp130).[4] The extracellular ligand binding domain of the leptin receptor isoforms are similar at the amino terminus but are varying at the carboxy terminus. The five isoforms Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd and Ob-Rf contain transmembrane domain, but intracellular motifs that activate signal transduction pathways are seen only in Ob-Rb isoform.[89] Ob-Re isoform lacks both transmembrane and intracellular domains and it has been found to circulate as soluble receptors.

Ob-Rb has been demonstrated in immune cells, T cells, NK cells, macrophages, polymorphonuclear cells, as well as neurons and intestinal epithelial cells.[90] Leptin binds to the receptors and affect the physiological and metabolic functions by the activation of JAK/STAT signalling pathways. The short type receptor (Ob-Ra, Ob-Rc, Ob-Rd) has been found to be expressed in the choroid plexus, lungs, kidneys and liver. They help in leptin transport into brain tissue and also in the degradation and removal of leptin. The soluble receptor Ob-Re combine with the leptin in the blood circulation. It has been found that the Ob-Ra, Ob-Rb and the Ob-Re secreted by the placenta play a major role in leptin transportation and for it's action in fetus and placenta. [91]

LEPTIN TRANSPORT AND SITES OF ACTION:

Though leptin is synthesized predominantly by adipocytes, it has been detected in low levels in the hypothalamus, pituitary, skeletal muscle, stomach, mammary epithelia, chondrocytes and a variety of other tissues. Leptin is partially bound to plasma proteins in the circulation.

Leptin activates the neurons of arcuate, ventromedial and dorsolateral hypothalamic nuclei and also the brain stem neuronal circuits regulating the feeding behaviour and energy balance.[92]Brain microvessels express high levels of Ob Ra receptors and help in binding and internalising leptin.[93]Cerebrospinal fluid also can transport leptin into the brain through the Ob-Ra receptors which are highly expressed in choroid plexus.

LEPTIN AND BODY FAT MASS:

Body weight is a non invasive predictor of body fat and caloric balance. Body mass index or "Quetelet Index" is a good index of body fat in both men and women. Serum leptin concentration correlates well with BMI in humans.[67] The mean serum leptin concentration is approximately four times higher in normal obese individuals than in lean healthy subjects. For a given BMI, the leptin levels in women is approximately twice that in men and this has been attributed to the testosterone.[75]

LEPTIN & FEEDING BEHAVIOUR:

Leptin has been found to bind to membrane receptors in the various regions of the brain, particularly in the arcuate nucleus of the hypothalamus. One set of neurons expresses orexigenic (appetite stimulating) peptides, Neuropeptide Y (NPY) & Agouti related peptide (AgRP). Leptin decreases the appetite by inhibiting the NPY and AgRP neurons, thus preventing the synthesis of orexigenic peptides. During fasting due to decrease in the leptin

concentration there is stimulation of NPY and AgRP production that results in increased appetite.

The second set of neurons containing the leptin receptors express proopiomelanocortin (POMC). Leptin binds to its receptors on the POMC neurons and make POMC to yield a variety of signal molecules, one among is Melanocyte stimulating hormone (MSH). MSH activates anorexigenic neurons and thus suppresses the appetite. On the other hand fasting inhibits MSH activity and stimulates eating. [94]

" Leptin is a bidirectional signal that switches physiological regulation between the fed and starved states."[95]

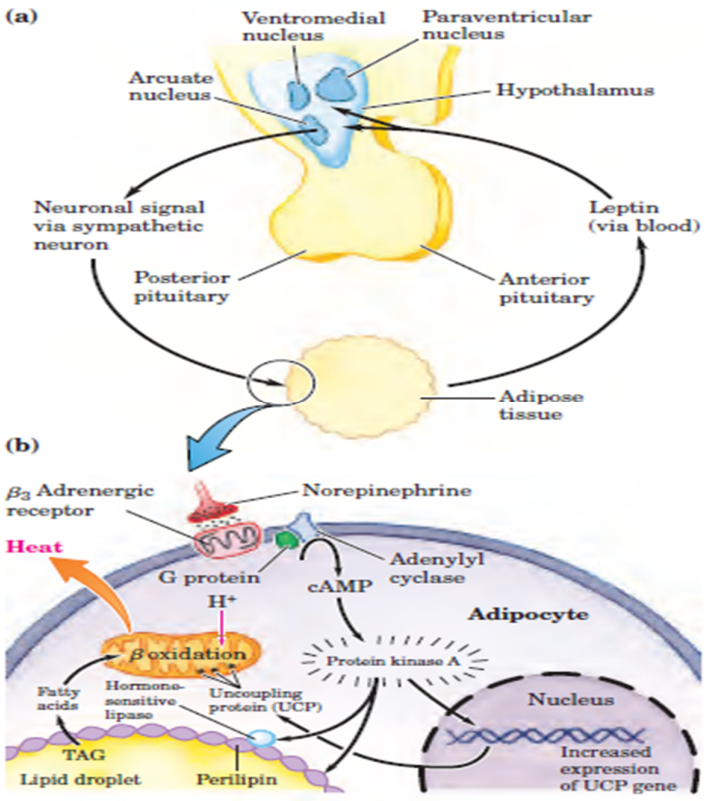


Figure 5 : Courtesy Williams textbook of endocrinology 12th edition

LEPTIN IN ENERGY BALANCE:

Leptin exerts its action on the sympathetic nervous system also. It increases the blood pressure, heart rate and also thermogenesis by uncoupling the mitochondria of white adipocytes. There is increased synthesis of the mitochondrial uncoupling protein thermogenin, a product of UCP 1 gene in adipocytes. Leptin alters the synaptic transmissions of the neurons in the arcuate nucleus to adipose and other tissues and stimulates thermogenin synthesis. Leptin causes an increased release of norepinephrine in these tissues, which act through β3 adrenergic receptors and stimulate transcription of UCP1 gene. Thermogenin or UCP forms a channel in the inner mitochondrial membrane allowing protons to re-enter the matrix of the mitochondria without passing through the ATP Synthase complex. This leads to the continuous oxidation of fatty acids in an adipocye without ATP synthesis, dissipiating energy as heat and consumption of dietary calories or stored fats in large amounts.[96]

Leptin infusion stimulates UCP-1 gene expression in brown adipose tissue, UCP-2 in white adipose tissue and UCP-3 in skeletal muscle and thereby increases energy expenditure.[97]

LEPTIN IN CARBOHYDRATE AND LIPID METABOLISM:

Leptin is known for its role in the long term regulation of bodyweight, but independent of this role it exerts acute effects on metabolism. For example, it has been observed in the ob/ob mice leptin decreases glucose and insulin

levels acutely even before there is detectable weight loss and also stimulation of glucose metabolism and gluconeogenesis in wild type rodents.[98,99,100]

Leptin has been found to stimulate lipolysis.[101] By its inhibitory effect on acetyl Co A carboxylase, it reduces the fatty acid and triglyceride synthesis and thereby decreases the intracellular concentration of lipids.[102] Thus leptin has beneficial effects on β cell function and insulin resistance and thereby improves glucose homeostasis.[101]

LEPTIN AND INSULIN:

Insulin secretion alike leptin depends on the body fat reserves and current energy balance. The insulin receptors in the appetite stimulating neurons of the arcuate nucleus inhibit the release of NPY and the insulin receptors present on the appetite suppressing neurons stimulate α MSH synthesis thereby reduces fuel intake and increases thermogenesis.

Leptin sensitizes the liver and muscle cells to insulin. This effect is explained by one hypothesis which states, "cross talk between the protein, tyrosine kinases activated by leptin and those activated by insulin; common second messengers in the two signalling pathways allow leptin to trigger some of the same downstream events that are triggered by insulin, through Insulin Receptors Substrate -2 (IRS-2) and Phospho Inositide 3-Kinase (PI-3K)."

The insulin receptor has intrinsic tyrosine kinase activity. When the leptin receptor is occupied by its ligand, phosphorylation occurs by a suitable

tyrosine kinase (JAK). The interaction between leptin and insulin is explained by the observation that both may phosphorylate the same substrate IRS-2, which activates PI-3K and thereby trigger the downstream consequences which include inhibition of food intake. IRS-2 acts as an integrator of the input from two receptors.[103]

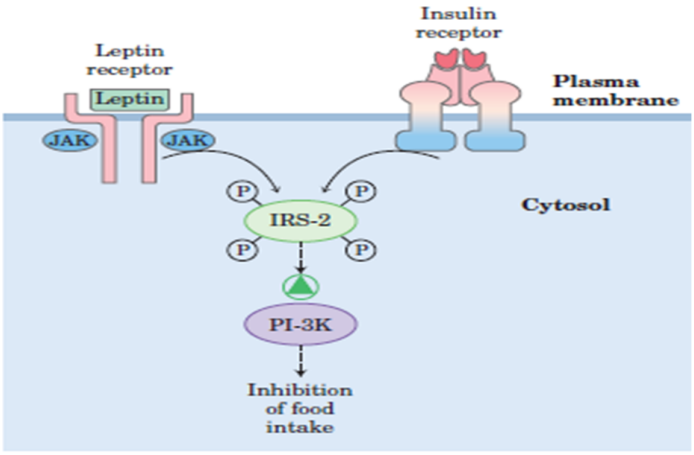


Figure 6 : Courtesy Lehninger Principles of biochemistry 5th edition.

LEPTIN AND GROWTH HORMONE:

Leptin plays a significant role in the regulation of body fat mass, regulating food intake and energy expenditure, and thereby can act as a metabolic signal regulating growth hormone (GH) secretion.[104]

Leptin and NPY producing hypothalamic neurons synapse with the neurons of somatostatin and antisera to NPY and somatostatin reverse starvation induced GH release. [105]

In hypopituitary adults with GH deficiency leptin concentration was found to be higher than could be expected for their body fat mass.[106]

LEPTIN AND THYROD HORMONE:

Rodent studies have shown that the fall in leptin levels associated with fasting can lead to thyroid axis suppression. In hypothyroid rodents low levels of leptin was observed. Infusion of leptin into the cerebral ventricles reversed some of the metabolic changes seen in hypothyroidism, for example improving glucose and reducing skeletal muscle fat.[107] However human studies on leptin and thyroid hormones showed contradicting results.[108]

LEPTIN AND OTHER HORMONES:

On fasting when the leptin level falls there is rise in glucagon, epinephrine and glucocorticoids. The net effect of these adaptations stimulate neoglucogenesis and provide glucose for vital cellular function and fatty acids for the skeletal muscle.[109] Studies have also shown that leptin is capable of regulating the hypothalamic-pituitary adrenal axis.[110]

LEPTIN IN PUBERTY AND REPRODUCTION:

Leptin has also been observed to regulate positively the hypothalamic-pituitary gonadal axis. This adipokine secreted by the peripheral adipocytes, plays a pivotal link between body fat and reproduction by signalling energy availability centrally.[111] Leptin receptors are expressed on GnRH secreting neurons and it has been found that leptin accelerates GnRH pulsatality.[112]

The serum leptin levels in blood is tightly correlated with the body fat stores and a threshold level is required for the activation of the hypothalamic-

pituitary gonadal axis. When serum leptin level increased during weight gain in treated patients with anorexia nervosa, it correlated positively with rise in gonadal steroids, testosterone, luteinizing hormone and follicle stimulating hormone. [113]

The ob/ob mice, which lack leptin and the db/db mice, which lack leptin receptors were obese and exhibited hypogonadotropic hypogonadism and this provided an evidence for the significant role played by leptin in reproduction. There was partial reversal of hypogonadism when recombinant leptin was administered to the ob/ob mice and to rats having pubertal delay due to food restriction.[114]

Serum leptin levels are increased during pregnancy and they are required for the growth of the fetus. Leptin secreted by the human placenta is identical to that produced by the adipose tissue. Energy deprivation is recognised rapidly than energy excess in pregnancy by the serum leptin concentration.[109]

LEPTIN AND HAEMATOPOIESIS:

Leptin plays a significant role in haematopoiesis and this can be explained by the following observations in various studies:

- Cytokine nature of leptin and it's receptors.
- Leptin receptor identification in the haematopoietic tissues.
- Adipocytes are the most abundant stromal cell type in adult human bonemarrow.[115]

Therefore it was thought that leptin in addition to serving as a local energy stores also played an important role in the regulation of haematopoiesis. Studies have also shown that leptin stimulate fetal and adult erythroid and myeloid series. It appears that the proliferating effect of leptin is mediated through the leptin receptor isoform, Ob-Rb, by JAK-STAT pathway.[116]

LEPTIN RESISTANCE AND OBESITY:

"The failure to respond to anorexigenic effects of leptin is called leptin resistance."[117] This leptin resistance could lead one to obesity.

Recent studies suggest that a group of proteins called Suppressors Of Cytokine Signalling play a major role in leptin resistance. These proteins by inhibiting receptor actions alter some hormone systems.

Insulin signalling pathways are initiated by autophosphorylation of tyrosine residues in the insulin receptor by leptin stimulation. SOCS proteins bind to the phosphorylated tyrosine residues on receptors or other members of the signal transduction pathway, and thereby disrupt signal flow and alter the cell's biochemical activity.[117]

Other mechanisms involved in leptin resistance include the following:

- Dysregulation in leptin synthesis and/or secretion.
- Abnormalities in leptin transport in brain.
- Abnormalities in leptin receptor and/or postreceptor signalling.[118]

Leptin transport in CSF may be limited in obesity and this is evidenced in obese individuals by decreased plasma:CSF leptin ratio. Studies have also shown that leptin resistance can be mediated by theSH-2 containing tyrosine phosphatase, SHP-2, as leptin signalling is enhanced when the binding site on Ob-Rb for SHP-2 is mutated.[119]

Leptin interacts with other metabolic signals, especially insulin and glucocorticoids in the regulation of body weight. These hormones are involved in the feeding behaviour and body weight regulation by regulating the expression of similar neuropeptides in the brain.

LEPTIN AND IMMUNE RESPONSE

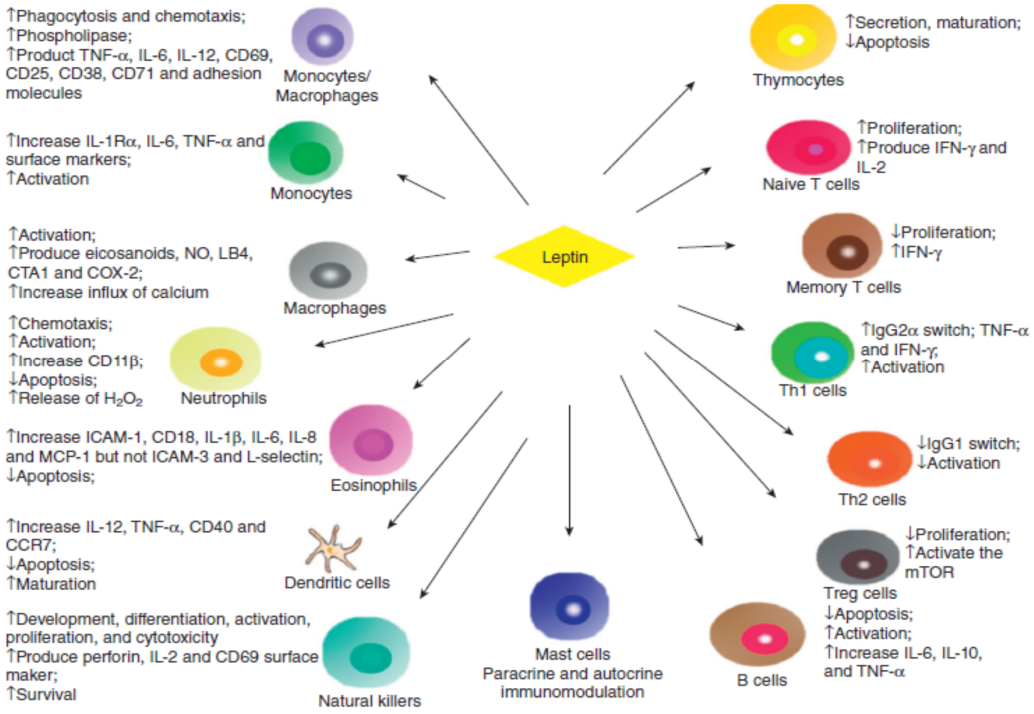


Figure 7 : Courtesy Tian et al 2014

Leptin has wide regulation on immune response. It regulates the innate immunity by promoting phagocytic functions and inducing eicosanoid synthesis. It also influences nitric oxide and several proinflammatry cytokines synthesis by macrophages and monocytes.[120] In murine macrophages, leptin increases IFN-γ induced expression of nitric oxide synthase. Leptin induces chemotaxis and release of reactive oxygen species. Finally leptin causes proliferation, differentiation, activation and cytotoxicity on natural killer(NK) cells.[121]

Leptin can also act on adaptive immune response. Adaptive immunity is classically divided into T helper 1(T_H1) and T helper 2(T_H2) pattern of cytokine secretion. $T_H1$cells synthesize proinflammatory cytokines which is essential for macrophage activation and cell mediated responses, whereas T_H2 lymphocytes produce anti-inflammatory modulators. Therefore for the optimal adaptive immune response there must be an adequate balance between T_H1 and T_H2 cell responses. Growth, differentiation and also T cell activation are influenced by leptin's interaction with T-cell co-stimulating antigens such as CTLA-4 and dipeptidyl peptidase. Glucocorticoid induced thymocytes apoptosis is prevented by leptin and thereby increases thymic cellularity.[122]

Leptin activates T cell and modifies T cell cytokine production pattern towards T_H1 response.[123] In leptin deficient individuals there was reduced number of CD4+ T cells with impaired T cell proliferation and cytokine release. Leptin receptors expressed on the human dendritic cells downregulate

their IL-10 production and drive the naive T cell proliferation towards a T_H1 phenotype. Thus from the above observations it is obvious that leptin plays a stimulatory role in adaptive immune response and favour T_H1 polarisation.

The immune suppression observed in leptin deficient (ob/ob) mice, in starvation and in reduced calorie intake has been found to be due to leptin deficiency which reflects low amounts of stored energy in the adipose tissue. This impaired immune response was found to be reverted by exogenous leptin administration.[124]

LEPTIN AS PROINFLAMMATORY MARKER:

Leptin regulates several cytokine secretion. It has been demonstrated that different inflammatory stimuli, like IL-1, IL-6 or lipopolysaccharide(LPS) can regulate mRNA expression as well as circulating leptin levels.[125] Furthermore, leptin is also produced by regulatory cells, suggesting that leptin expression could trigger or participate in the inflammatory process through direct, para or autocrine actions.

The reason behind the leptin dependent resistance in the development of innate immune mediated inflammation is unknown, but there are reports of imbalance between pro and anti- inflammatory cytokines,[126] suggesting leptin mediated cytokine secretion in monocytes /macrophages through STAT 3 activated pathway.[127] In leptin deficient adaptive immune mediated inflammation models, an imbalance between T_H1 and T_H2 lymphocytes was

observed, which resulted in altered cytokine secretion leading on to resistance to inflammation.

Recent studies have shown elevated serum leptin levels in acute ulcerative colitis patients where it activates the nuclear factor –κB. Moreover it has been demonstrated that serum leptin levels are negatively correlated with CD4+ and CD25+ regulatory T cells suggesting that leptin might have a major implications in the pathogenesis of different autoimmune disease s which is charecterised by T_H1 autoreactivity.[128]

LEPTIN AND SIGNALLING PATHWAYS:

Leptin binds to its receptor and brings about various biochemical and molecular mechanisms through certain signalling pathways. JAK-STAT pathway is one of the main signalling cascades and others are MAPK/ERK, AMPK and P13K.

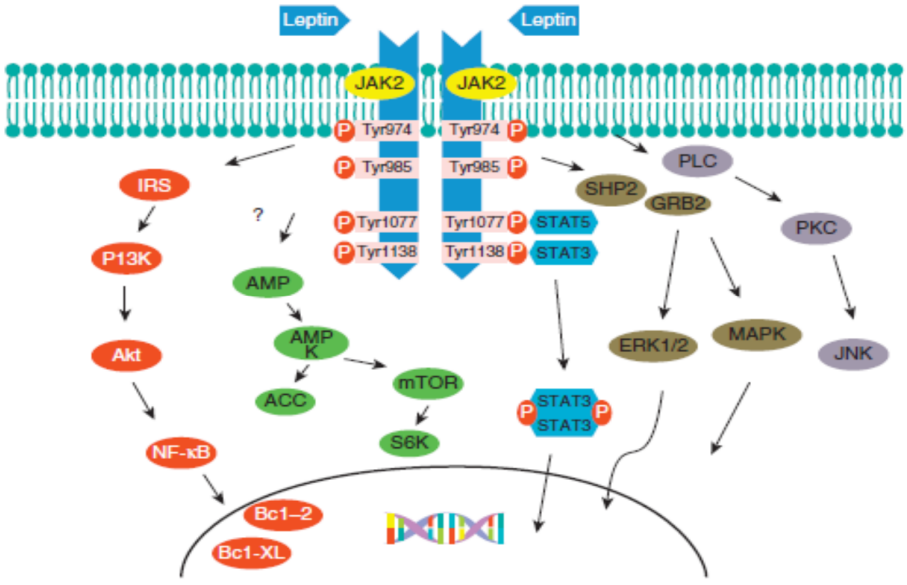


Figure 8 : Courtesy Tian et al 2014

i)JAK-STAT Pathway:

When leptin binds to leptin receptors it activates the JAK2 pathway by phosphorylating the tyrosine residues on the leptin receptor and induces the STAT-3 and STAT-5 pathway. The phosphorylated STAT-3 dimers enter the nucleus and function as transcription factors in the expression of targeting genes like SOCS3 and neuropeptides.[129]

ii) MAPK/ERK Pathway:

After the phosphorylated tyrosine residues recruitment of SHP2, it activates the MAPK pathways, such as ERK1/2, p38 MAPK and p42/44 MAPK, by its interaction with the GRB2 protein, which then enter the nucleus and mainly mediate chemokine and cytokine genes.[130]

iii) P13K/Akt Pathway:

This is an important signalling pathway for leptin required for inducing immune and inflammatory responses. This pathway regulates various functions which includes the following:

- Regulates anti-apoptotic transcription factor NF-κB in the production of various genes such as Bcl-2 and Bcl-XL.[131]
- Akt can suppress the functions of transcription factor Fox01or FKHR-L1 which are involved in the production of Bim from Bcl-2 family proteins and also in the direct regulation of leptin functions in the hypothalamus.

- Stimulates insulin signalling such as IRS1/2 and participate in glucose metabolism.[132]

iv)AMPK Pathway:

Studies have proved that leptin is involved in the regulation of CaMKK2/AMPK/ACC and the mTOR/ribosomal S6K pathways, thereby influencing the carbohydrate and lipid metabolism.[133]

iv)Other Pathways:

When leptin binds to OB-Rb it stimulates PLC, which in turn activates c-Jun N-terminal protein kinase by activating PKC. It has also been reported that this PLC/PKC signalling system can act in the pancreatic islets in insulin release.

LEPTIN AND RHEUMATOID ARTHRITIS:

Most studies have shown that interaction between neuroendocrine and immune systems lead to the pathogenesis of RA. Among the many neuroendocrine mediators, leptin is known to play a major role in the development of RA and in many studies elevated serum leptin levels has been demonstrated. Leptin regulates development, proliferation, apoptotic maturation and activation both in innate and adaptive immunity leading to inflammatory changes.

In RA patients as a proinflammatory cytokine, leptin increases IL-6 production by activating JAK/STAT pathway.[134] Studies have indicated that it

also inhibits androstenedione secretion, which is anti-inflammatory, and this can perpetuate chronic inflammatory diseases.[135] Seven et al observed that the serum and synovial fluid leptin levels were higher in RA patients when compared to the control group.

Among the multiple connective tissues found in the normal joint, the articular cartilage is the most affected in rheumatic diseases. The chondrocytes maintains a delicate balance between the synthesis and degradation of the extracellular matrix and under pathological conditions this balance becomes altered and a host of inflammatory mediators are produced by the chondrocytes leading to complete loss of articular cartilage. It has also been reported leptin along with IFN-γ and IL-1 induces NOS type II activation in the chondrocytes[5]by the signalling pathways that involves PI-3 Kinase, MEK-1 and p38 Kinase and produces nitric oxide which is a well known proinflammatory mediator in the joint cartilage triggering chondrocyte phenotype loss, apoptosis and metalloproteases activation. Therefore from the increased levels of leptin in the serum, synovial fluid and cartilage it is obvious that leptin plays a key role in the pathogenesis of RA.

Thus when the literatures were reviewed the significance of Leptin in immune regulation and autoimmunity was understood. Therefore this study was carried out to know about the Leptin status in RA patients so that future therapeutic measures can be decided in the treatment of RA.

Aims & objectives

AIM OF THE STUDY

The aim of the study is to determine the concentration of leptin in the serum of Rheumatoid arthritis patients and to correlate it with the disease activity in those patients.

The objectives of the study are

- To compare the serum leptin concentration in rheumatoid arthritis patients with that of the apparently healthy individuals.

- To correlate serum leptin levels with that of erythrocyte sedimentation rate, C- reactive protein and body mass index.

MATERIALS AND METHODS

This study was conducted after obtaining ethical committee approval from institutional ethics committee, Madras Medical college held on 20.01 2015. A copy of the approval is enclosed. Volunteers were examined only after obtaining informed consent. A copy of the information sheet and the informed consent regarding the study is enclosed .

STUDY DESIGN : Case – Control Study

STUDY CENTRE: Institute of Biochemistry and Department of Rheumatology, Madras Medical College.

STUDY PERIOD : January 2015 – September 2015

STUDY SUBJECTS SELECTION

CASES : 60 Rheumatoid arthritis patients diagnosed by the Rheumatologists in the Rheumatology OPD according to American College of Rheumatology (ACR) criteria 2010.

INCLUSION CRITERIA:

1. Rheumatoid arthritis patients diagnosed by American College of Rheumatology (ACR) criteria 2010 with symptoms of early morning stiffness of more than 1 hour , symmetrical joint involvement with pain, tenderness and swelling in the joints.
2. Age 25-55years

EXCLUSION CRITERIA:

1. Patients with Diabetes Mellitus, Hypothyroidsm, Hypertension, Coronary Vascular Disease.
2. Patients on Estrogen & Statins.
3. Pregnancy and lactation.

DIAGNOSTIC CRITERIA FOR RHEUMATOID ARTHRITIS

ACR / European League Against Rheumatism (EULAR) Criteria 2010 :

Diagnostic criteria for Rheumatoid arthritis

The diagnosis is based on clinical and serological variables. The total score has to be more than or equal to 6 for a diagnosis of Rheumatoid arthritis.

Joint involvement:

Small joints:- metacarpophalangeal joints, proximal interphalangeal joints, the interphalangeal joint of the thumb, second through fifth metatarsophalangeal joint and wrist.

Large Joints:- shoulders, elbows, hip joints, knees, and ankles .

- 1 large joint - 0 point
- 2–10 large joints - 1 point
- 1–3 small joints (with or without involvement of large joints) - 2 points
- 4–10 small joints (with or without involvement of large joints) - 3 points
- More than 10 joints (with involvement of at least 1 small joint) - 5 points

Serological Parameters:

Rheumatoid factor and ACPA (anti-citrullinated protein antibody)

- Negative RF *and* negative ACPA 0 points
- Low-positive RF *or* low-positive ACPA 2 points
- High-positive RF *or* high-positive ACPA 3 points

Acute phase reactants

Elevated erythrocyte sedimentation rate (ESR) / elevated CRP (c-reactive protein) value - 1 point

Duration of arthritis

For symptoms lasting six weeks or longer - 1 point

CONTROLS: Age and sex matched apparently healthy volunteers among the students and staff of Madras Medical College.

SAMPLE COLLECTION AND PROCESSING

5 ml of peripheral venous blood was collected from the antecubital vein of these patients by applying tourniquet. Blood samples were collected from the patients around 9.00 am to avoid diurnal variation of leptin in fasting state and for uniformity in the collection. Serum was separated after clot formation and by centrifugation at 3000 rpm/min for 10 minutes. Serum was aliquoted and stored at -20˚C in the deep freezer until leptin was analysed. Other analytes were assayed as the samples were collected.

PARAMETERS ASSESSED

1. Serum Leptin.
2. C-Reactive protein (CRP).
3. Rheumatoid factor (RF).
4. Serum Uric acid
5. Serum Fasting Lipid profile.
6. Serum Urea, Serum Creatinine
7. Erythrocyte sedimentation rate(ESR**)**
8. Haemoglobin

CALCULATED PARAMETERS

1. **BMI:- calculation**

Body mass index was calculated using "Quetelet Index".

BMI = W (Weight in Kg) / H^2 (Height in m)^2

Grades of BMI

Normal	-	18.5 – 24.9
Overweight	-	25 – 29.9
Obese	-	30 and above

2. **DAS 28(3) – calculation**

Disease activity score was calculated using the following formula

$$DAS = [0.56*\sqrt{T28} + 0.28*\sqrt{S28} + 0.7*\ln(ESR)]* 1.08 + 0.014* GH$$

T- no of tender joints, S- no of swollen joints, GH – General Health

Grades of Disease activity

< 3.2	low activity
3.2 – 5.1	moderate activity
> 5.1	high activity

SERUM LEPTIN ESTIMATION

METHOD: ELISA- Sandwich method

Kit Manufacturer : DRG

PRINCIPLE

1. Anti Leptin antibodies are coated on the microtitre wells.
2. Test sera are applied.
3. The sample is incubated with a specific biotinylated monoclonal antileptin antibody and forms a sandwich complex.
4. After incubation the unbound material is washed off and enzyme complex is added to detect the bound leptin.
5. After incubation the unbound materials are again washed away.
6. The substrate, TMB solution is added, which produces a color change by the action of the horse radish peroxidase enzyme present in sandwich complex. The intensity of the color developed is proportional to the concentration of the Leptin in the test sera.
7. The reaction is stopped by the addition of dilute Sulphuric acid.
8. Absorbance is measured at 450 nm and the concentration is directly proportional to the colour developed.

MATERIALS REQUIRED

1. Anti- Leptin monoclonal antibody coated microtitre plate – 96 wells
2. Assay buffer.
3. Antiserum containing monoclonal biotinylated anti-Leptin antibody.
4. Enzyme complex of strepavidin conjugated to horse radish peroxidase.
5. Substrate solution – Tetramethyl benzidine.
6. Stop solution –0.5M Sulphuric acid (H_2SO_4)
7. Standards:

Std 0	0 ng/mL	Lyophilised human serum free of Leptin.
Std 1	2 ng/mL	Leptin diluted in human serum – lyophilised.
Std 2	5 ng/mL	
Std 3	25ng/mL	
Std 4	50 ng/mL	
Std 5	100 ng/ mL	

8. Controls - Low - 2.5- 6.6ng/mL High – 24.4 – 45.4ng/mL
9. Wash solution.
10. Calibrated micropipettes - 15µL, 50 µL, 100µL.
11. Deiodinised water.
12. Disposable pipette tips.
13. Absorbent paper.
14. Microtitre plate reader with 450 nm

Reagent preparation

1. All reagents and the microtitre wells strip were brought to room temperature.
2. 0.5 mL of distilled water was added to lyophilised content of standard vials and allowed to stand for 10 minutes. Mixed gently several times before use.
3. Control vials containing the lyophilised contents were reconstituted by adding 0.5mL of deiodinised water and allowed to stand for 10 minutes. Before use it was mixed gently for several times.
4. Wash solution was prepared by adding 30 mL of concentrated wash solution with 1170 mL of distilled water to make it up to 1200mL.
5. All other reagents were ready for use.

PROCEDURE

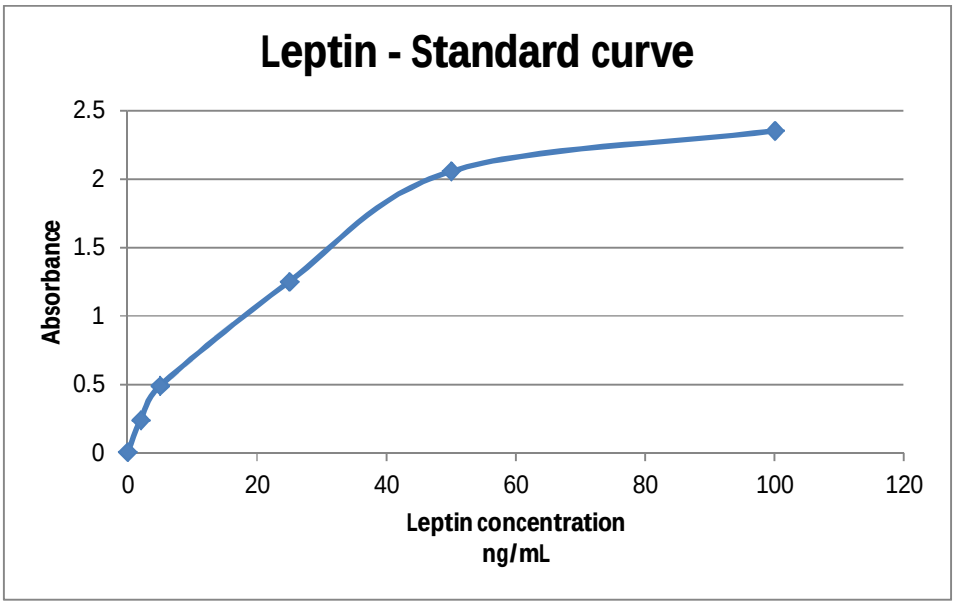
1. 15 µL of standard were pipetted in the1st 6 wells and study subjects serum added in the remaining wells.
2. 100 µL of assay buffer added in all wells, mixed for 10 seconds.
3. Incubated for 120 minutes at 20 -25˚c.
4. Briskly shake out the contents of the wells.
5. Machine washing was done 3 times using distilled wash solution, 300 µL wash solution was used per cycle for each well. The remaining fluid present in the wells were removed by striking the wells against absorbent paper.
6. 100 µL of antiserum was added to each well.
7. Incubated for 30 minutes at room temperature.
8. Briskly shake out the contents of the wells.

9. The wells were rinsed by the machine 3times using 300 µL of wash solution per cycle for each well. The remaining fluid present in the wells were removed by striking the wells against absorbent paper.
10. 100 µL of enzyme complex was added to each well.
11. Incubated for 30 minutes at room temperature.
12. The wells were rinsed by the machine 3times using 300 µL of wash solution per cycle for each well. The remaining fluid present in wells were removed by striking the wells against absorbent paper.
13. 100 µL of substrate solution was added in each well, gently shaken for 5 seconds.
14. Incubated in dark for 15 minutes at room temperature.
15. 50 µL of stop solution was added in each well to stop the enzymatic reaction and gently shaken for 30 seconds.
16. Optical density was measured at 450nm in ELISA reader within 10 minutes after adding the stop solution.

Calculation of results

1. The standard curve is plotted using concentration along x – axis & absorbance along y- axis

Leptin concentration ng/mL,	absorbance
0	0.0070
2	0.2386
5	0.4893
25	1.2527
50	2.0584
100	2.3552



Using the absorbance values for each sample the concentration is calculated with the help of standard curve.

Reference value : Male : 3.84 ± 1.79ng/mL

Female : 7.36 ± 3.73ng/mL

C- REACTIVE PROTEIN(CRP) ESTIMATION (Quantitative method)

METHOD: Turbidimetric test.

KIT USED: AVITEX TURBO CRP

PRINCIPLE: The latex particles are coated with antibodies to human CRP. When the diluted is with mixed with samples containing CRP agglutination causes an absorbance change that can be quantified by comparing to a calibrator of known CRP concentration.

REAGENTS:

Latex: Suspension of polystyrene latex particles coated with goat Ig G antihuman CRP-concentrate.

Diluent: Tris buffer pH 8.2. Working strength.

Calibrator: Calibrator serum containing CRP concentration of 55mg/L – lyophilized.

Reagent preparation:

Calibrator: Reconstitute with addition of 1mL of deiodinised water.mix thoroughly. Do not allow to foam. Bring to room temperature before use.

Working reagent:1mL concentrated latex + 9mL diluent.

ASSAY PROCEDURE:

1. Bring the working reagent and photometer cuvette holder to 37˚C.
2. Semi autoanalyser was programmed for estimating the CRP by using the following parameters.

Mode	-	Fixed time
Wavelength	-	540nm (530- 550nm)
Temperature	-	37˚C.
Units	-	mg/L
No. of calibrators/standards	-	1
Concentration of calibrator	-	55mg/L
Blank	-	Deiodinised water
Sample volume	-	5µL
Reagent volume	-	1000 µL
No. of readings	-	2
Delay time secs	-	5 secs
Readtime	-	120 secs
Normal low	-	0 mg/L
Normal high	-	6 mg/L
Linearity limit	-	150 mg/L

Prepare sample:

1.Pipette into a cuvette 1mL of working reagent.

2. Add 5 µL of sample.

3.Mix and read the absorbance immediately.(A_1Sample)

Prepare calibrator:

4.Pipette into a cuvette 1mL of working reagent.

5. Add 5 µL of calibrator.

6. Mix and read the absorbance immediately. (A_1Calibrator)

7. Incubate at 37˚C.

8.After 2 minutes read the absorbance of the sample (A_2Sample) and the calibrator (A_2Calibrator).

Results and Interpretation:

$$\frac{(A_2\text{Sample}) - (A_1\text{Sample})}{(A_2\text{Calibrator}) - (A_1\text{Calibrator})} \times \text{Concentration of Calibrator} = \text{CRP mg/L}$$

Normal value upto 6mg/L.

RHEUMATOID FACTOR (Semi Quantitative method)

METHOD: Latex Agglutination

KIT USED: ACCUCARE

PRINCIPLE: Rheumatoid factor present in serum agglutinates the latex particles coated with human gamma globulin. If the rheumatoid factor level in serum is greater than 8 IU/mL, agglutination will occur.

MATERIALS REQUIRED

1. RF Latex reagent
2. Positive control
3. Negative control
4. Glass slides
5. Stirrer rods
6. Droppers

PROCEDURE

1. 1 drop of serum,1 drop of positive control, 1 drop of negative control are added in different circles on the slide
2. RF Latex reagent is added in all the circles.
3. Stirrer is used to mix and spread the contents in test circle.
4. The slide is rotated and observed for agglutination within 2 minutes.
5. The samples showing agglutination are positive for Rheumatoid factor.
6. These samples are quantitated by diluting with normal saline.
7. Samples in these dilutions 1:2, 1:4, 1:8, 1:64 are prepared and subjected to the above described method until there is no agglutination.

CALCULATION

RF = Highest dilution with positive reaction * 8 IU/mL (reagent sensitivity)

ESTIMATION OF SERUM CREATININE (Auto analyser)

METHOD: Modified Jaffe's reaction.

KIT USED: Erba Mannheim XL System Packs

PRINCIPLE: Creatinine reacts with alkaline picrate to produce a reddish orange colour. This is a non specific reaction.

REAGENT COMPOSITION

R1

Sodium hydroxide	240 mmoles/L

R2

Picric acid	26 mmoles/L.

CALIBRATION: Done with serum based XL multical calibrator.

ASSAY PARAMETERS:

Primary wavelength	505 nm
Secondary wavelength	570 nm
Assay type	Rate A
Curve type	Linear.
R1 volume	160 µL
R2 volume	40 µL
Sample volume	10 µL.

CALCULATION : Results are calculated automatically by the instrument.

<u>Reference values:</u>

Males: 0.7-1.4mg/dL.

Females: 0.6-1.2mg/dL.

ESTIMATION OF SERUM UREA (Autoanalyser)

METHOD: Urease – Glutamate dehydogenase.

KIT USED: Erba Mannheim XL System Packs

PRINCIPLE:

Urea is hydrolysed by urease enzyme to ammonia and carbon dioxide in the presence of water.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} NH_3 + CO_2$$

Ammonia combines with alpha ketoglutarate in the presence of NADH to give glutamate & NAD

$$NH_3 + \alpha\text{ ketoglutarate} + \text{NADH} \xrightarrow{\text{Glutamate dehydrogenase}} \text{L- Glutamate} + \text{NAD}$$

The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to formation of NAD.

REAGENT COMPOSITION:

R1

Tris buffer	100mmol/L
Alpha – ketoglutarate	5.49 mmol/L
Urease (Jack Bean)	≥ 10KU/ L
GLDH (microorganism)	≥ 2.5KU/ L

R2

NADH	1.66 mmol/L

CALIBRATION: Done with serum based XL multical calibrator.

ASSAY PROCEDURE:

Primary wavelength	340 nm
Secondary wavelength	415 nm
Assay type	Rate A
Curve type	Linear
R1 volume	160µL
R2 volume	40µL
Sample volume	2µL

CALCULATION: Results are calculated automatically by the instrument.

Reference range: 15-50 mg/dL.

ESTIMATION OF SERUM URIC ACID

METHOD: Uricase – POD. Trinder reaction.

KIT USED: Erba Mannheim XL System Packs

PRINCIPLE:

Uricacid in the presence of uricase is oxidised to allantoin. Peroxidase reacts with H_2O_2 catalyses the oxidative coupling of 4AAP with TOOS to form Quinoneimine complex with the intensity of color is proportional to the concentration of uric acid in sample.The absorbance of final colour measured at 546nm is proportional to the concentration of uric acid in the sample.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$\text{TOOS} + 4\text{AAP} + 2\,H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4\,H_2O.$$

REAGENT COMPOSITION:

R1 + R2

Pipes buffer (pH 7.0)	50mmol/L
TOOS	0.48 mmol/L
Uricase	≥ 200U/ L
Peroxidase	≥ 5000U/ L
4 – Aminoantipyrine	1.5 mmol/L

CALIBRATION: Done with serum based XL multical calibrator.

ASSAY PROCEDURE:

Primary wavelength	546 nm
Assay type	End point
Curve type	Linear
R1 volume	160µL
R2 volume	40µL
Sample volume	4µL

CALCULATION: Results are calculated automatically by the instrument.

Reference Values:

Men: 3.6-7.7mg/dL.

Women: 2.5-6.8mg/dL.

ESTIMATION OF HDL CHOLESTEROL (Semiautoanalyser)

METHOD: Phosphotungstic acid method, Endpoint assay.

PRINCIPLE:

Phosphotungstate precipitates Chylomicrons, LDL and VLDL in the presence of divalent cations. The supernatant ,which contains HDL cholesterol remains unaffected and is estimated using cholesterol reagent.

Serum $\xrightarrow{\text{Phosphotungstate}}$ HDL + (Chylomicrons, LDL, VLDL Precipitate).

PRECIPITATING REAGENT:

Phosphotungstic acid	2.4mmol/L.
Magnesium chloride	40mmol/l.

PROCEDURE:

1) Precipitation: Precipitation of LDL, VLDL, Chylomicrons. 500µL of precipitating reagent & 250µL of sample is mixed well and allowed to stand for 10 minutes at room temperature. Centrifuge for 10 minutes at 4000 r.p.m to get a clear supernatant. Concentration of HDL cholesterol is determined by using cholesterol reagent.
2) Estimation with Cholesterol reagent: 50µL of supernatant is pipetted into 1000µL of Cholesterol working reagent and incubated for 10 minutes at 37□C. Read the absorbance at 505 nm.

CALCULATION:

HDL Cholesterol=Absorbance of test / Absorbance of Std x concentration of Std x 2 mg/dL (2 is the dilution factor due to deproteinisation step).

REFERENCE VALUES:

In Male	: 35-55mg/dL
In Females	: 45-65mg/dL.

ESTIMATION OF TOTAL CHOLESTEROL (Autoanalyser)

METHOD : Cholesterol esterase-Cholesterol Oxidase.

KIT USED : Erba XL System packs.

PRINCIPLE: Cholesterol esterase hydrolyses Cholesterol ester to Cholesterol free fatty acids. .It is acted upon by Oxidase to form cholest-4 en- 3- one and Hydrogen peroxide. Hydrogen peroxide reacts with aminoantipyrine and phenol to give pink coloured complex and its optical density measured at 540nm.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acids.

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholest-4-en-3-one + H_2O_2

2 H_2O_2 + 4AAP + Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine dye + 4 H_2O.

REAGENT PREPARATION:

Good's buffer	50mmol/L
Cholesterol oxidase	$\geq$50 U /L
Cholesterol esterase	$\geq$ 200U/L
Peroxidase (Horseradish)	$\geq$ 1200U/L
4-Aminoantipyrine	0.3mmol/L
Phenol	5 mmol/L

CALIBRATION: Done with serum based XL multical calibrator.

ASSAY PROCEDURE:

Primary wavelength	505 nm
Secondary wavelength	700 nm
Assay type	Endpoint

Curve type	Linear
Reagent volume	200µL
Sample volume	2 µL

CALCULATION: Results are calculated automatically by the instrument.

Reference interval:150 - 260mg/dL.

ESTIMATION OF TRIGLYCERIDES (Autoanalyser)

METHOD: Glycerol-3-phosphate Oxidase (GPO) Method.

KIT USED: Erba XL System packs.

PRINCIPLE: Triglycerides (TGL) in the sample is hydrolysed by Lipoprotein Lipase to give glycerol and free fatty acids. Glycerol is then acted upon by glycerol kinase to form glycerol-3-phosphate, which on action by oxidase is converted into DHAP & hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine to give pink coloured complex. The intensity of colour is related to concentration of Triglyceride in the sample.

$TGL + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol + Free Fatty acids.}$

$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-3-Phosphate + ADP}$

$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol-3-phosphate oxidase}} \text{DAP} + H_2O_2$

$H_2O_2 + \text{4AAP + TOOS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 2\ H_2O$

REAGENT PREPARATION:

R1

PIPES buffer (pH 7.0)	50 mmol/L

ATP	2.85 mmol/L
Mg	60 mmol/L
Glycerol kinase (GK)	≥ 1.5 U/mL
Glycerol-3-phosphate oxidase (GPO)	≥ 6.0 U/mL

R2

Peroxidase	≥ 15.0 U/mL
Lipoprotein Lipase	≥ 25.0 U/mL
4-Aminoantipyrine	1.5 mmol/L

CALIBRATION: Done with serum based XL multical calibrator.

ASSAY PROCEDURE:

Primary wavelength	546 nm
Secondary wavelength	700 nm
Assay type	Endpoint
Curve type	Linear
R1	160 µL
R2	40 µL
Sample volume	2 µL

CALCULATION: Results are calculated automatically by the instrument.

Reference Values: Males: 60-165mg/Dl, Females: 40-140mg/dL.

Erythrocyte Sedimentation Rate : Measured by conventional Westegren Method

Haemoglobin : Estimated by automated cell counter.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software version 22 and the following were carried out

- Tests of significance at 5% significance using Unpaired student's t-test was done to compare the Serum Leptin levels between cases and controls.
- Pearsons correlation co-efficient was done to measure the linear relationship between serum Leptin concentration and other parameters like DAS28(3), CRP, ESR and BMI.
- One Way - ANOVA Analysis of Variance to compare more than 2 variables in the same group & between groups was carried out to compare serum Leptin concentration between four groups viz- RF & CRP positive, RF positive & CRP negative, RF negative & CRP positive, RF & CRP negative.

Results

MASTER CHART FOR CASES

S.no.	Age	Sex	Duration	Menstrual hty	Ht	Wt	BMI	ESR	RF	CRP	Leptin	DAS28(3)	TC	TGL	HDL	LDL	Hb	BP	UA	Urea	Creati
1	55	M	1yr		160	60	23.44	50	Pos	12.49	12.93	5.57	137	84	60	60.2	11.2	130/80	10.3	32	0.9
2	45	F	10yrs	Menopause	148	55	25.11	35	Neg	4.69	21.22	5.37	154	199	39	75.2	10.2	100/80	4.3	30	1.1
3	44	F	3yrs	Reproductive	154	59	24.89	25	Pos	15.85	41.56	4.25	150	153	45	74.4	10.9	110/80	4.9	27	1
4	53	F	10yrs	Menopause	162	71	27.09	40	Pos	34	82.77	4.46	145	160	63	50	11.9	140/80	4.5	27	0.8
5	50	M	1.5yrs		148	48	21.9	48	Pos	13.75	38.29	5.11	166	141	54	83.8	12.6	100/70	8.2	28	0.9
6	38	F	2yrs	Reproductive	152	67	29.1	15	Pos	12.58	45.92	4.21	163	90	78	67	11.4	130/80	4	25	0.8
7	55	F	3yrs	Menopause	158	92	36.8	18	Pos	24	92.62	4.84	189	285	60	72	12.5	120/80	8.5	30	1.1
8	52	M	8yrs		162	57	21.75	24	Pos	20.6	56.53	5.23	224	194	51	134.2	11.8	100/60	4.5	36	1
9	43	F	2yrs	Menopause	140	48	24.48	15	Neg	5.6	54.56	4.33	179	207	54	83.6	10.8	110/70	4.9	18	0.8
10	45	F	2yrs	Reproductive	148	60	27.39	20	Pos	38	88	5.04	178	104	66	91.2	8.5	130/80	4.2	46	1.6
11	48	M	1yr		170	60	20.76	40	Pos	27.22	18.541	5.07	156	130	66	64	13.6	120/70	7.2	18	0.8
12	54	F	3yrs	Menopause	140	54	27.55	100	Pos	27.65	82.17	6.6	122	97	42	60.6	11.4	120/70	3.9	26	0.8
13	36	F	2yrs	Reproductive	145	53	25.22	30	Pos	9	11.48	5.95	144	106	78	44.8	10.8	100/70	4.5	20	0.7
14	41	F	20yrs	Reproductive	139	55	28.55	40	Pos	4.12	12.95	5.6	201	135	45	129	11.8	110/70	3.2	29	1.1
15	32	F	2yrs	Reproductive	148	58	26.5	20	Pos	38.12	93.11	5.49	195	146	51	114.8	12.4	110/70	6.6	22	1.3
16	36	F	4yrs	Reproductive	145	39	18.57	46	Pos	18.72	5.28	6.28	145	122	39	81.6	10.2	110/70	4.3	26	1.1
17	43	F	8yrs	Menopause	148	71	32.42	82	Pos	15.52	98.12	6	174	113	36	115.4	12.4	150/80	5.1	28	1.2
18	32	F	12yrs	Reproductive	155	70	29.16	38	Pos	16.12	45.56	5.15	184	156	54	98.8	10.6	100/80	6.1	30	1.1
19	34	F	6yrs	Reproductive	145	39	18.57	42	Pos	40.57	16.58	4.8	164	214	27	94.2	9.8	90/60	6.9	21	0.9
20	42	F	2yrs	Menopause	155	63	26.25	47	Pos	18.12	58.54	4.89	215	155	36	148	12.4	100/70	3	19	1
21	40	M	1yr		160	76	29.68	26	Pos	13.02	16.96	5.02	167	152	69	67.6	11.5	120/80	5.6	25	1.3
22	45	F	3yrs	Menopause	155	51	21.25	48	Pos	12.91	13.84	5.43	207	181	63	107.8	12.8	130/80	4.4	29	1.1
23	30	F	3yrs	Reproductive	148	70	31.96	78	Pos	18.12	58.59	6.2	205	93	78	108.4	11.9	110/70	4.8	25	0.9
24	45	F	15yrs	Menopause	140	44	22.44	20	Neg	13.48	53.71	4.69	205	92	54	132.6	10.6	100/70	5.9	32	1.2
25	43	F	11yrs	Menopause	147	60	27.77	98	Pos	12	15.63	6.85	182	97	42	120.6	10.5	130/80	6.4	19	0.8
26	45	M	2yrs		158	71	28.4	42	Pos	26.51	88.56	5.66	166	100	42	104	13.8	130/80	6.4	21	0.7
27	28	F	3yrs	Reproductive	152	54	23.38	35	Pos	13	24.09	5.51	210	85	42	151	8.1	100/70	5.8	26	0.9
28	40	F	7yrs	Reproductive	147	66	30.55	35	Pos	36	96.24	5.03	217	132	57	133.6	12	100/70	6.7	26	1.1
29	32	F	11yrs	Reproductive	158	70	28	20	Neg	16.15	93.18	5.61	206	93	54	133.4	12.8	110/80	12.9	29	1
30	41	F	3yrs	Reproductive	157	71	28.8	10	Neg	13.04	49.18	4.16	270	105	36	213	11.2	110/80	3.4	23	0.8
31	50	F	5yrs	Menopause	140	58	29.6	35	Neg	27	12.31	5.01	154	89	72	64.2	10.9	110/80	6	30	1.2

S.no.	Age	Sex	Duration	Menstrual hty	Ht	Wt	BMI	ESR	RF	CRP	Leptin	DAS28(3)	TC	TGL	HDL	LDL	Hb	BP	UA	Urea	Creati
32	32	M	10yrs		157	45	18.75	15	Pos	24.99	96.12	4.99	190	92	45	126.6	11.8	110/70	5.9	25	0.9
33	38	F	10yrs	Reproductive	150	59	26.22	15	Pos	19.87	75.56	5.51	210	102	66	123.6	12.2	140/80	6.1	28	1.1
34	32	F	12yrs	Reproductive	156	65	26.75	22	Pos	14.68	58.85	5.06	168	99	42	106.2	11	130/80	3.2	29	0.9
35	35	F	4yrs	Reproductive	148	54	24.65	38	Pos	18	88.45	5.91	183	217	30	109.6	12.4	100/70	5.3	28	1.1
36	45	F	6yrs	Menopause	150	64	28.4	40	Pos	5.8	16.14	5.82	219	211	51	125.8	12.2	130/80	6.3	28	1.3
37	40	F	2yrs	Reproductive	145	66	31.42	10	Pos	5.6	49.18	3.5	254	105	48	185	14	100/70	3.9	24	0.8
38	45	F	5yrs	Reproductive	152	68	29.43	15	Neg	15.02	15.65	3.92	215	231	30	138.8	11.8	110/70	7.2	23	0.9
39	32	F	8yrs	Reproductive	150	52	23.11	55	Pos	17.34	16.64	4.9	141	80	33	92	12.4	120/70	2.8	24	0.8
40	41	F	4yrs	Reproductive	139	48	24.9	25	Neg	5.74	39.56	4.51	181	99	24	137.2	10.2	100/60	4.3	26	0.9
41	39	F	2yrs	Reproductive	148	41	18.72	20	Neg	12.93	13.53	4.2	171	105	36	114	9.8	90/60	3.9	22	0.9
42	42	F	5yrs	Reproductive	152	58	25.1	65	Pos	4.6	27.17	5.03	193	206	33	118.8	10.4	110/70	4.6	26	0.8
43	44	F	3yrs	Menopause	150	68	30.22	25	Neg	22.35	96.54	5.03	160	279	81	23.2	11.2	110/80	5.2	28	0.9
44	35	F	5yrs	Reproductive	153	56	24.34	40	Neg	11.3	21.98	4.7	150	224	69	36.2	11.6	100/70	4.6	21	0.9
45	42	F	7yrs	Reproductive	150	62	27.55	35	Pos	24.12	98.16	5.09	200	176	63	101.8	12.8	110/80	5.3	28	1.1
46	40	F	2yrs	Reproductive	145	54	25.7	15	Pos	12.2	95.81	5.1	187	106	48	117.8	11.9	100/70	3.2	25	0.9
47	46	F	14yrs	Reproductive	157	58	23.57	55	Pos	10.4	54.86	5.71	153	95	63	71	9.8	100/70	4.1	36	1.3
48	40	F	10yrs	Reproductive	155	56	23.33	20	Neg	18.26	80.17	4.45	145	108	78	45.4	12.2	110/80	3.1	29	1.1
49	45	F	5yrs	Reproductive	150	52	23.11	35	Pos	5.9	34.03	4.49	198	86	66	114.8	11.8	130/80	4.3	26	0.9
50	47	F	10yrs	Reproductive	140	55	28.06	35	Pos	14.08	32.73	5.53	220	145	54	137	10.5	100/70	6.2	28	1.2
51	55	F	3yrs	Menopause	160	82	32.03	22	Neg	20.06	89.12	5.2	207	104	36	150.2	11.6	140/80	4.3	32	0.9
52	35	F	1.5yrs	Reproductive	158	78	31.33	37	Pos	23.19	91.65	5.29	192	273	33	104.4	10.2	100/70	3.6	19	0.7
53	29	M	4yrs		157	67	27.24	52	Pos	14.54	19.21	4.6	208	299	39	109.2	12.6	120/70	7.8	22	0.8
54	33	F	6yrs	Reproductive	145	45	21.43	36	Pos	10.4	21.08	5.72	170	52	33	126.6	9.8	90/60	4	20	0.9
55	35	F	4yrs	Reproductive	165	52	19.26	10	Neg	12.03	15.72	3.81	181	103	36	124.4	10.6	100/60	2.8	20	0.8
56	25	F	1yr	Reproductive	160	52	20.31	15	Pos	31.92	26.17	3.99	95	108	30	43.4	11	100/70	4.3	18	0.7
57	26	F	3yrs	Reproductive	152	60	26	45	Pos	34.63	93.18	5.38	204	140	54	122	12.4	120/70	3	21	0.8
58	45	F	8yrs	Reproductive	165	60	22.05	15	Pos	13.6	32.75	4.3	196	186	33	125.8	11.2	110/80	3.6	28	1.1
59	35	F	4yrs	Reproductive	138	36	18.94	20	Pos	12.12	12.01	4.14	193	239	63	82.2	9.6	90/60	4	24	0.9
60	50	F	4yrs	Menopause	148	64	29.22	15	Pos	3.61	15.62	4.02	115	176	51	28.8	10.4	140/80	3.4	29	0.9

MASTER CHART FOR CONTROLS

S.No.	Age	Sex	Menstrual Hty	Ht	Wt	BMI	ESR	RF	CRP	Leptin	TC	TGL	HDL	LDL	Hb	BP	Uricacid	Urea	Creatinine
1	50	F	Reproductive	154	68	28.7	16	neg	3	29.18	186	160	42	112	11.6	100/70	4.8	24	0.9
2	42	M		155	65	26.75	12	neg	5.2	19.55	184	256	39	93.8	12.4	130/70	5.6	22	0.8
3	27	M		158	65	26.1	10	neg	4.8	13.08	163	177	57	70.6	14.2	110/70	8.1	16	0.5
4	36	M		158	63	25.3	8	neg	5.7	10.6	104	87	33	53.6	13.6	110/80	5.9	18	0.9
5	32	M		165	62	22.8	10	neg	1.1	3.99	170	137	66	76.6	13.8	110/80	8.8	15	0.8
6	40	F	Reproductive	164	66	23.54	6	neg	4.2	10.5	150	92	69	62.6	12.8	100/70	3	21	0.9
7	40	F	Reproductive	153	61	26.5	14	neg	5.9	13.85	197	120	60	113	13.4	110/80	3.7	22	0.8
8	36	F	Reproductive	150	83	36.39	22	neg	5.2	18.4	174	138	36	110.4	14.6	120/70	6	17	0.9
9	33	F	Reproductive	145	58	27.62	13	neg	4.9	15.44	183	71	42	126.8	12.8	100/70	6	16	0.7
10	41	F	Reproductive	148	61	27.85	22	neg	3.6	12.19	198	140	48	122	13.6	140/80	4.3	15	1.1
11	45	F	Reproductive	150	61	27.11	15	neg	2.8	7.58	256	182	57	162.6	12.8	110/70	4.5	16	0.8
12	55	M		166	82	29.7	20	neg	3.1	5.99	220	275	33	132	13.8	130/80	11.3	22	1.2
13	31	F	Reproductive	148	64	29.2	22	neg	3.8	9.34	193	140	63	102	11.8	100/70	4	18	0.8
14	36	F	Reproductive	158	80	32.13	12	neg	5.7	12.54	161	179	36	89.2	13.8	120/70	4.4	16	0.7
15	24	F	Reproductive	152	65	28.14	18	neg	5.3	10.58	138	252	33	54.6	12.4	120/80	6	19	0.9
16	43	F	Reproductive	160	58	22.7	8	neg	3.2	6.44	168	115	39	106	11.6	110/70	4.5	15	0.8
17	48	F	Menopause	162	86	32.82	17	neg	4.8	5.56	135	216	36	55.8	11.2	150/80	6.2	25	0.9
18	41	F	Reproductive	154	66	27.8	12	neg	2.9	7.26	184	196	39	105.8	10.8	110/70	5.1	20	0.9
19	42	F	Reproductive	155	81	33.75	9	neg	3.6	10.43	175	99	69	86.2	11.8	100/70	7.7	18	1.1
20	41	F	Reproductive	157	75	30.49	10	neg	3.4	17.98	196	118	72	100.4	12.9	120/80	4.6	21	0.7
21	32	F	Reproductive	152	80	34.6	20	neg	3.8	10.82	190	157	69	89.6	13.4	120/80	7	15	0.8
22	32	F	Reproductive	150	71	31.55	13	neg	2.8	21.06	152	254	66	35.2	12.8	110/70	7.4	17	0.7
23	32	F	Reproductive	153	62	26.95	15	neg	1.4	7.25	228	133	78	123.4	10.69	90/60	5.8	15	0.7
24	36	F	Reproductive	153	66	28.7	21	neg	4.2	25.15	157	153	63	63.4	11.8	110/80	6.2	17	0.9
25	34	F	Reproductive	145	46	21.9	6	neg	1.8	7.35	134	90	66	50	12.2	100/70	5.6	20	1
26	45	F	Menopause	152	65	28.13	15	neg	4.6	14.31	128	98	66	42.4	11.4	110/80	2.7	25	1.1
27	52	F	Menopause	157	75	30.49	12	neg	5.6	11.56	207	152	66	110.6	11	110/80	5.6	28	1.2
28	43	F	Reproductive	140	65	33.16	15	neg	4.2	10.12	153	116	72	57.8	11.8	120/80	5	23	1
29	48	M		164	68	25.27	8	neg	1.8	4.41	164	143	42	93.4	13.4	120/80	6.2	26	1.2
30	41	F	Reproductive	150	58	25.77	12	neg	2.1	5.6	172	136	51	93.8	11.2	100/70	3.5	21	1.1

RESULTS

Table 1 Distribution of study population based on age

		Group					
		Case		Control		Total	
		N	%	N	%	N	%
Age group (yrs)	≤ 30	5	8.3	2	6.7	7	7.8
	31 – 35	13	21.7	7	23.3	20	22.2
	36 – 40	10	16.7	6	20.0	16	17.8
	41 – 45	20	33.3	10	33.3	30	33.3
	46 – 50	6	10.0	3	10.0	9	10.0
	> 50	6	10.0	2	6.7	8	8.9
	Total	60	100.0	30	100.0	90	100.0

Table1 shows that the major study population belongs to the 30 to 50 age group as observed in various studies.

Table 2 Distribution of study population based on gender

		Group					
		Case		Control		Total	
		N	%	N	%	N	%
Gender	Male	8	13.3	6	20.0	14	15.6
	Female	52	86.7	24	80.0	76	84.4
	Total	60	100.0	30	100.0	90	100.0

Table 2 shows the number of female and male patients in the study. This table shows that majority of the patients are female, and thus it indicates that women are more affected than men.

Table 3 Comparison of serum leptin levels between cases and controls in both males and females with RA

	Group	N	Mean	Std. Dev	SE Mean	t-Value	P-Value
Leptin	Case	60	48.7777	32.00198	4.13144	8.608	<0.001 S
	Control	30	11.9370	6.11431	1.11632		

S - Significant

Table 3 shows serum leptin concentration compared between cases and controls using unpaired student's t-test. The mean serum leptin concentration was 48.78±32.00 ng/mL in cases and 11.94±6.11 ng/mL in controls. The standard error of mean for cases was 4.13 and that of the controls was 1.12. The 95 % confidence interval for mean serum leptin concentration in the cases was 56.88 ng/mL to 40.68ng/mL. The 95% confidence interval for mean serum leptin concentration in the controls was 14.13ng/mL to 9.75ng/mL. The p-value obtained was less than 0.001 and it was statistically highly significant.

Figure1 Mean S.Leptin concentration in both females and males between cases and controls with RA

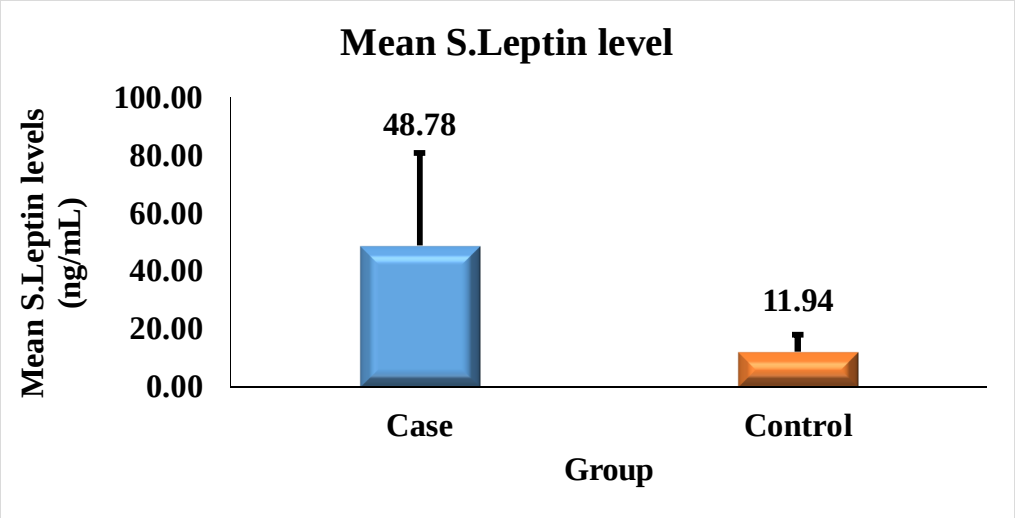


Table 4 Compare mean Serum Leptin concentration between cases and controls (Females)

Gender		Group	N	Mean	Std. Dev	t-Value	P-Value
Female	Leptin	Case	52	49.6062	32.02568	8.039	<0.001 S
		Control	24	12.5204	6.11400		

S – Significant

Serum leptin concentration among the females between cases and controls was compared using unpaired student‘s t-test. The mean leptin concentration in female patients with RA was 49.61± 32.03ng/mL and in controls it was 12.52± 6.11ng/mL. The difference in the mean between the two groups was statistically highly significant and the p-value was <0.001.

Figure 2 Mean serum leptin concentration in female patients with RA and female controls.

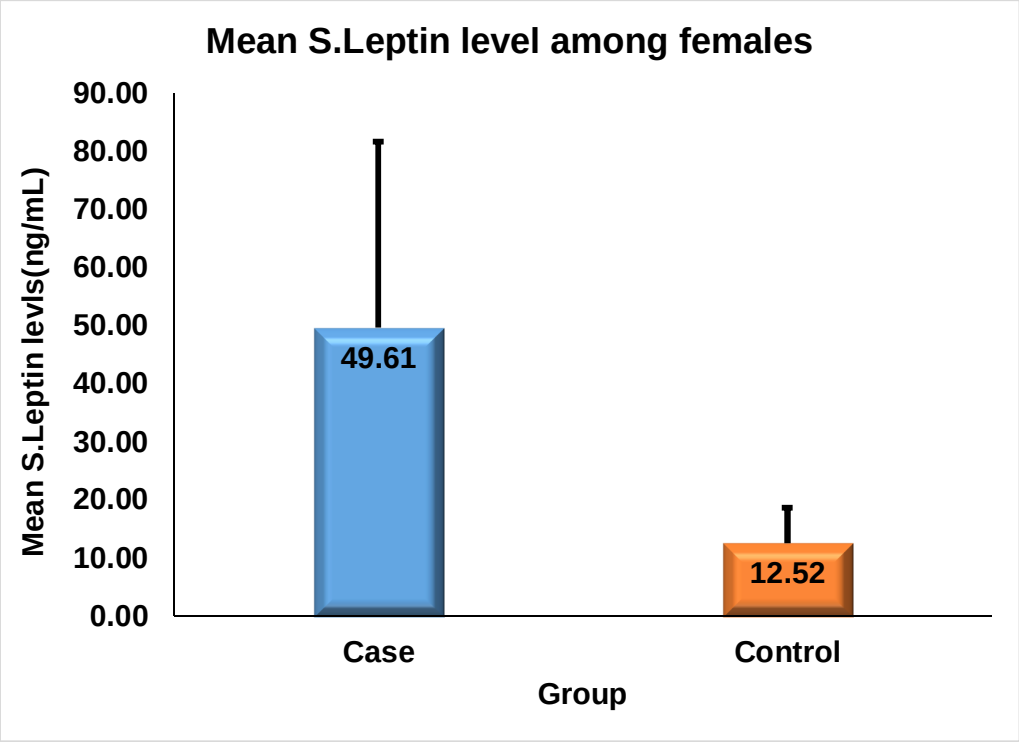


Table 5 Comparison of serum leptin concentration in males between cases and controls.

Gender		Group	N	Mean	Std. Dev	t-Value	P-Value
Male	Leptin	Case	8	43.3926	33.48337	2.794	0.025 S
		Control	6	9.6033	6.05893		

S - Significant

Table 5 compares the mean serum leptin concentration between the male RA patients and the male controls. The mean leptin concentration in male RA patients was 43.39±33.48ng/mL and in controls 9.60±6.06 ng/mL. The serum leptin concentration in both these groups was compared using student's t- test. The difference in the mean between the two groups was statistically significant and the p-value was 0.025.

Figure 3 Mean serum leptin concentration in male RA patients and male controls

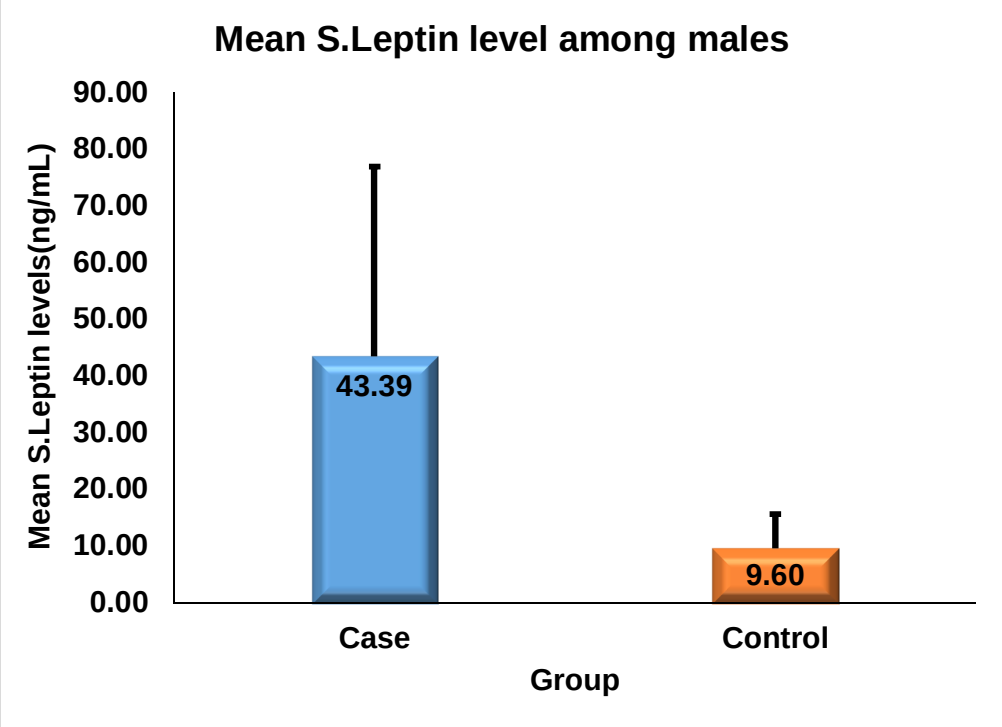


Table 6 DAS 28 (3) (Disease Activity Score) to grade disease activtity in patients with RA

Disease Activity Score	Case	
	N	%
Low Activity	0	0.0
Moderate Activity	34	56.7
High Activity	26	43.3
Total	60	100

Table 6 shows the percentage distribution of disease activity in RA patients. 56.7% of patients had moderate disease activity with a DAS Score of 3.2 to 5.1. 43.3% of the patients had high disease activity with a DAS Score of more than 5.1.

Figure 4 Disease activity in cases based on the DAS 28(3) in cases.

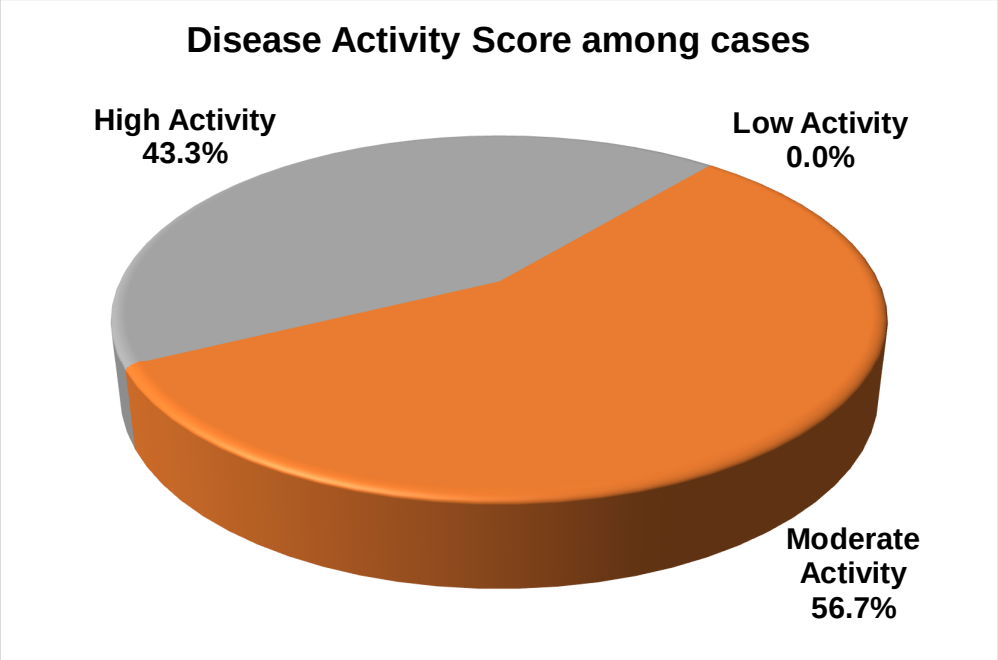


Table 7 Comparison of Mean S.Leptin Concentration among patients with High and Moderate Disease Activity Score

	Disease Activity Score	N	Mean	Std. Dev	t-Value	P-Value
Leptin	Moderate Activity	34	46.8341	30.92205	0.535	0.595 NS
	High Activity	26	51.3192	33.80734		

NS – Not Significant

Table 7 shows comparison of the mean serum leptin concentration among the RA patients with moderate and high activity using unpaired student's t –test. Though the mean serum leptin concentration in high activity (n=26) was more (51.32±33.81ng/mL) than that in moderate activity (n=34) (46.83±30.92ng/mL), the difference in the mean between the two groups was not statistically significant and the p-value was 0.595.

Figure 5 Mean serum leptin concentration in male and female RA patients with moderate and high disease activity

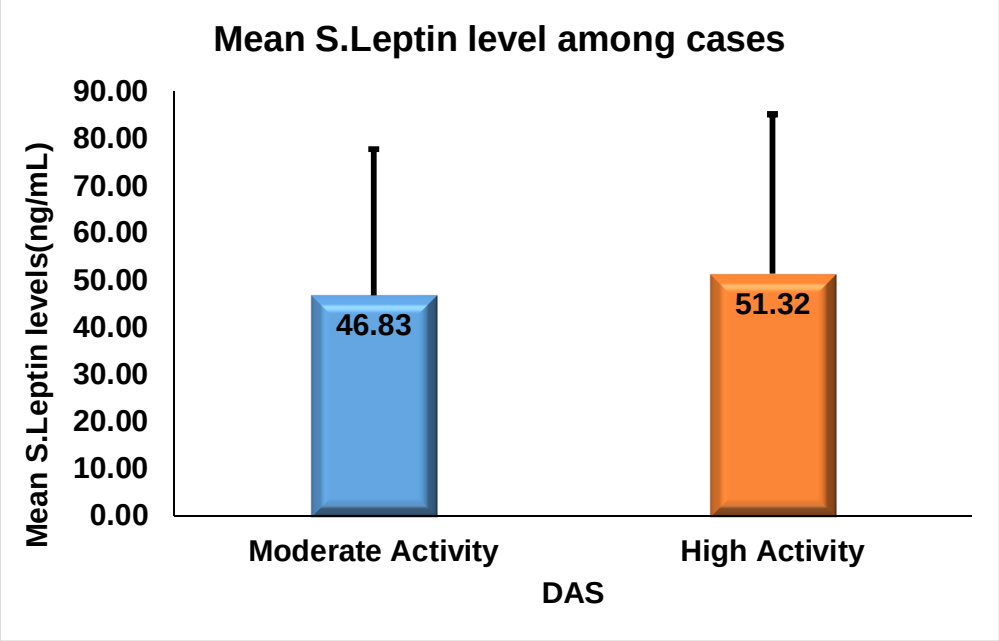


Table 8 Mean serum leptin concentration based on Body Mass Index in both patients and controls

BMI Level	Group	S.Leptin (ng/mL)			t-Value	P-Value
		Number	Mean	Std.Dev		
Normal (BMI 18.5-24.9)	Case	25	35.6	24.99	5.505	<0.001
	Control	4	7.1	2.69		
	Total	29	31.6	25.22		
Over wt (BMI 25-29.9)	Case	27	50.6	33.13	5.789	<0.001
	Control	17	12.4	6.86		
	Total	44	35.8	32.16		
Obese (BMI 30 & above)	Case	8	84.0	18.99	10.248	<0.001
	Control	9	13.2	4.95		
	Total	17	46.5	38.71		
Total	Case	60	48.8	32.00	8.608	<0.001 S
	Control	30	11.9	6.11		
	Total	90	36.5	31.56		

S - Significant

Table 8 shows the mean serum leptin concentration between the normal, overweight and obese groups of RA cases and apparently healthy controls. The mean difference was compared using student's t-test between the cases and controls, totally and also between the groups. The mean serum leptin concentration in the normal group was 35.6$\pm$24.99ng/mL in cases and 7.1$\pm$2.69ng/mL in controls, in the overweight group it was 50.6$\pm$33.13ng/mL in cases and 12.4$\pm$6.86ng/mL controls, in the obese group it was 84.0$\pm$18.99ng/mL in cases and 13.2$\pm$4.95ng/mL in controls and totally it was 48.8$\pm$32.0ng/mL in cases and 11.9$\pm$6.11ng/mL in controls. The mean difference between the cases and controls, totally and also between the groups was statistically significant with a p-value of <0.001.

Figure 6 Mean serum leptin concentration in normal, overweight and obese cases and controls

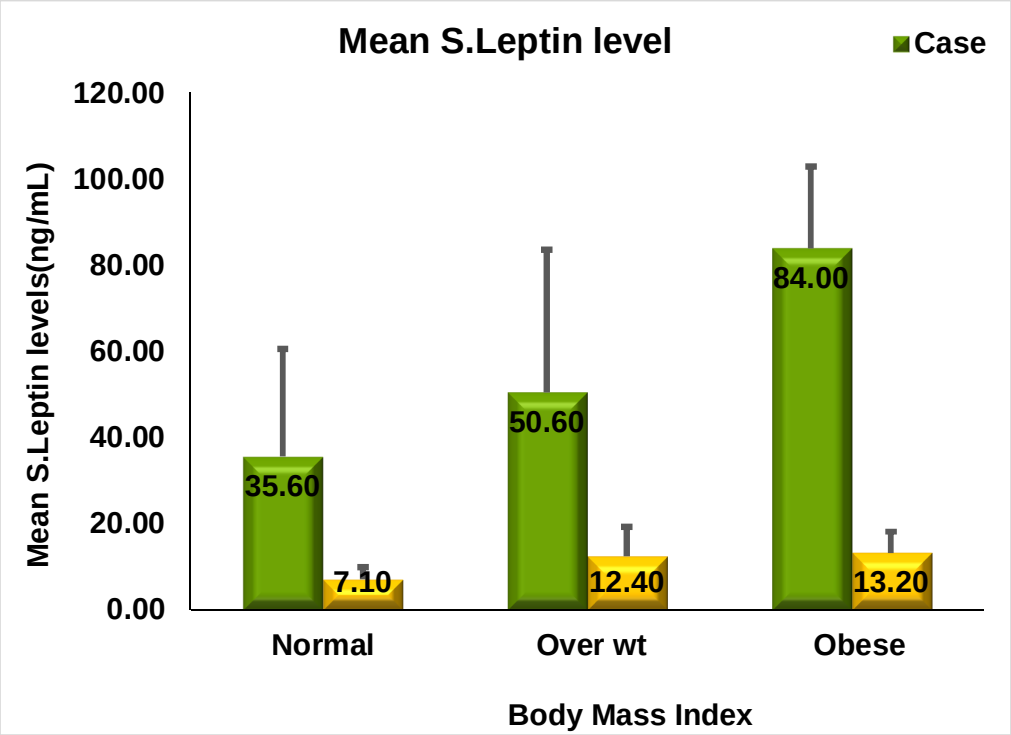


Table 9 To compare mean serum CRP concentration between patients with RA and healthy controls

	Group	N	Mean	Std. Dev	t-Value	P-Value
CRP	Case	60	17.2872	9.25046	11.043	<0.001 S
	Control	30	3.8167	1.36257		

S - Significant

Table 9 shows the mean CRP concentration between cases and controls. The mean serum CRP concentration in cases was 17.29±9.25 mg/L and that of controls was 3.82±1.36 mg/L. The serum CRP concentrations was compared in both these groups using unpaired student's t -test. The difference in the mean was statistically significant with a p-value of <0.001.

Figure 7 Mean serum CRP concentration in RA patients and healthy controls.

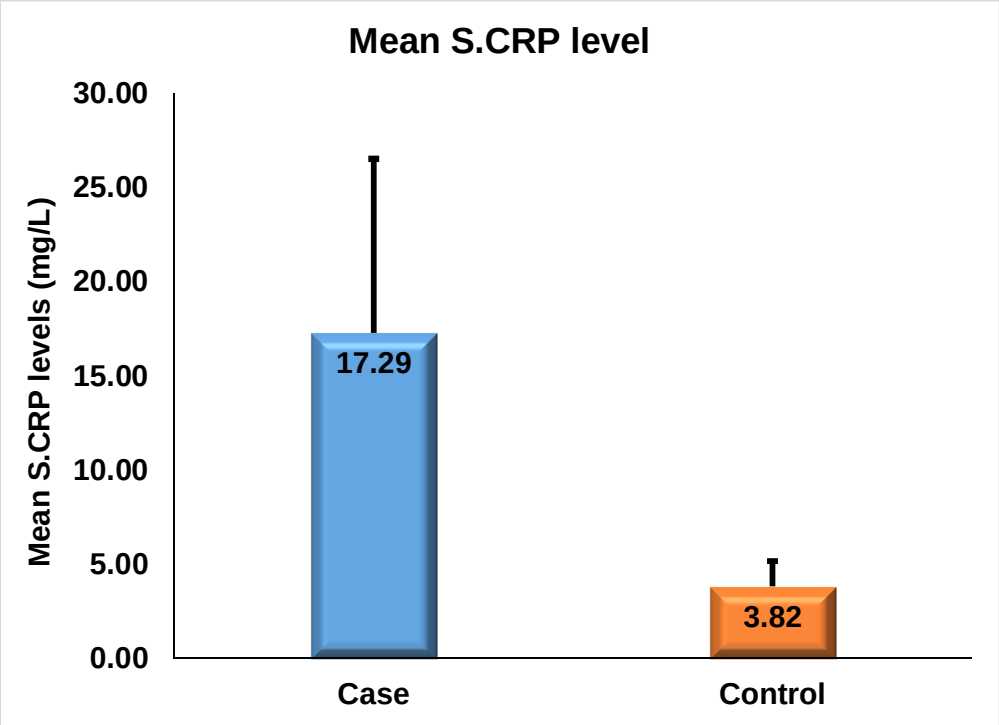


Table 10 Comparison of seum Leptin levels between CRP +v e & CRP –ve patients with RA

Group		Sr. CRP Level	N	Mean	Std. Dev	t-Value	P-Value
Case	Leptin	Positive	51	52.0830	33.12270	3.206	0.004 S
		Negative	9	30.0478	15.22072		

S - Significant

Table 10 shows the mean CRP concentration between the CRP +ve cases and CRP –ve cases with RA. The mean serum leptin concentration in CRP +ve cases was 52.08±33.12mg/L and that of CRP –ve cases was 30.05±15.22mg/L. The serum CRP concentration was compared in both these groups using unpaired student's t -test. The difference in the mean was statistically significant with a p-value of <0.004.

Figure 7 Mean serum CRP concentration in CRP+ve & CRP –ve RA patients

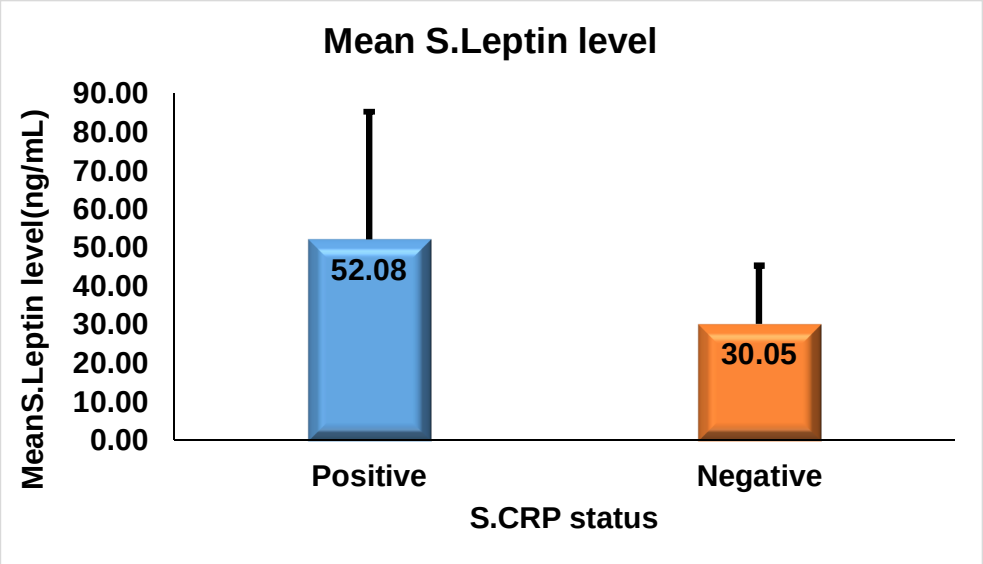


Table 11 shows the RF status in the patients with RA

RF	Number	%
Positive	46	76.7
Negative	14	23.3
Total	60	100.0

Table 11 shows the RF status in the RA patients. Patients with RF+ve was 76.7% and those negative was 23.3%.

Figure 8 shows the Rheumatoid factor status in the RA patients.

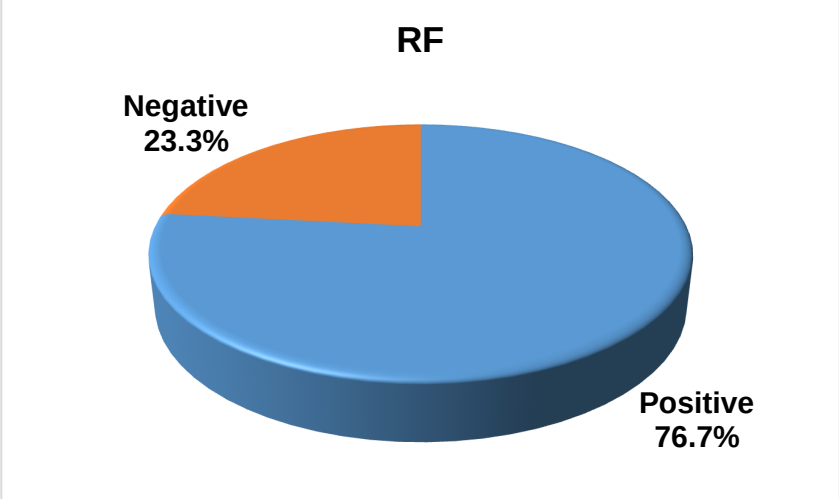


Table 12 Mean serum leptin concentration between RF+ve & RF-ve patients with RA

	RF	N	Mean	Std. Dev	t-Value	P-Value
Leptin	Positive	46	49.3528	32.37266	0.250	0.803 NS
	Negative	14	46.8879	31.86361		

NS – Not Significant

Table 12 shows the mean leptin concentration between the RF +ve cases and RF–ve cases with RA.The mean serum leptin concentration in RF +ve cases was 49.35±32.37ng/mL and that of RF–ve cases was 46.89±31.86ng/mL. The serum leptin concentrations was compared in both these groups using unpaired student's t -test. The difference in the mean was not statistically significant and the p-value was 0.803.

Figure 9 Mean serum leptin concentration in the RF +ve & RF –ve RA patients

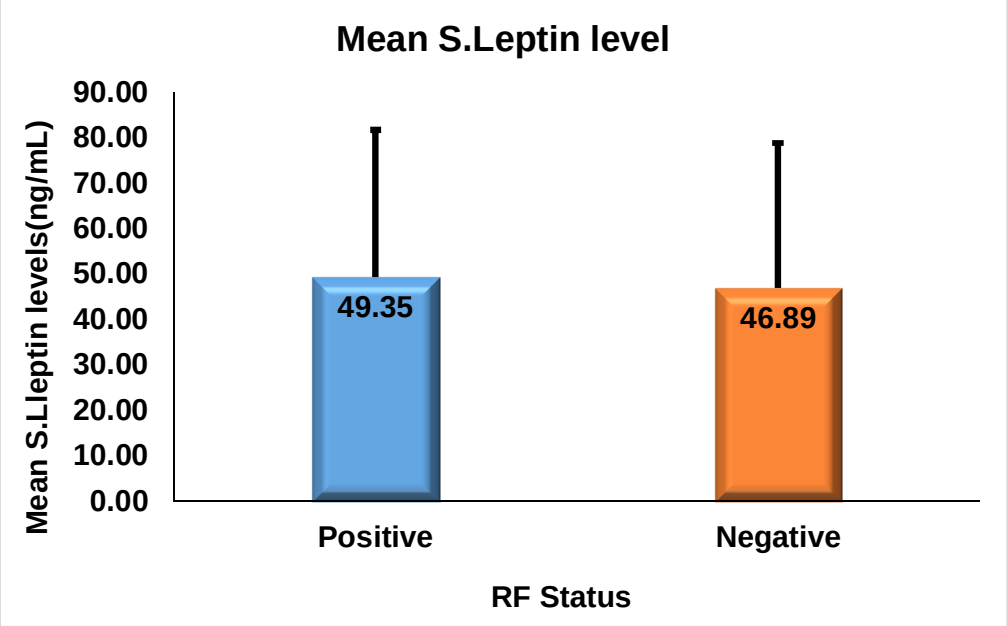


Table 13 One way ANOVA to compare leptin level in patients with RA between RF+ve & CRP +ve , RF+ ve & CRP-ve, RF-ve & CRP +ve, RF-ve & CRP –ve groups.

Table 13a Oneway ANOVA to compare mean values between groups.

Group		N	Mean Leptin	Std. Deviation	F-Value	P-Value
Case	CRP+ve & RF+ve	40	52.8785	32.96044	1.376	0.259
	CRP –ve & RF+ve	6	25.8483	13.98779		
	CRP +ve & RF-ve	11	49.1900	35.16968		
	CRP -ve& RF -ve	3	38.4467	16.69786		
	Total	60	48.7777	32.00198		

Table 13 b ANOVA Table

Group	**Sum of Squares**		**df**	**Mean Square**	**F-Value**	**P-Value**
Case	Between Groups	4149.266	3	1383.089	1.376	0.259
	Within Groups	56274.223	56	1004.897		
	Total	60423.490	59			NS

NS – Not Significant

Table 13 shows ANOVA was used to compare the mean serum leptin concentration between the 4 groups. The mean serum leptin concentration was higher in RA patients with both RF+ve and CRP+ve, than the other 3 groups viz, CRP+ve alone, RF+ve alone and both CRP and RF negative. The difference in the mean was not statistically significant; p-value 0.259.

Table -14 Comparison of mean ESR between patients with RA and healthy controls

	Group	N	Mean	Std. Dev	t-Value	P-Value
ESR	Case	60	34.48	20.001	7.593	<0.001 S
	Control	30	13.77	4.826		

S - Significant

Table 14 shows the mean ESR level between the cases with RA and controls. The mean ESR level in cases with RA was 34.48mm/hr and that of controls was 13.77mm/hr. The ESR level was compared in both these groups using unpaired student's t -test. The difference in the mean was statistically significant and the p-value was <0.001.

Figure 10 Comparison of mean ESR between cases and controls.

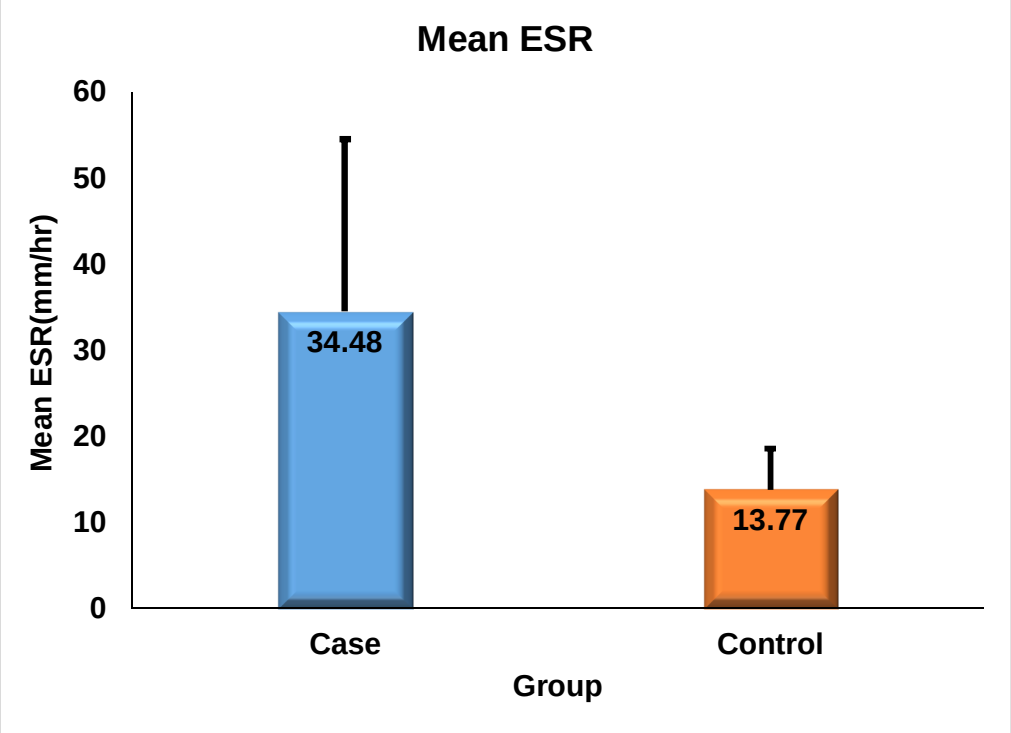


Table 15 Correlation between DAS 28(3) and ESR in RA patients

Group: Case		ESR
DAS 28(3)	Pearson Correlation	0.695
	P-Value	<0.001
	N	60

Table 15 shows the correlation between DAS 28(3) score and ESR in RA patients. Pearsons correlation coefficient was used to find the association between disease activity score and ESR. A moderate positive correlation was observed between DAS and ESR with r value of 0.695 and this was statistically significant with a p-value of <0.001.

Table 16 Correlation between DAS (28) and Leptin in RA patients

Group: Case		DAS 28(3)
Leptin	Pearson Correlation	0.154
	P-Value	0.240
	N	60

Table 16 shows the correlation between leptin and DAS in RA patients. Pearsons correlation coefficient was used to find the association between leptin and DAS. No correlation was observed between leptin and DAS (r =0.154) and this was not statistically significant.

Figure 11 Correlation between S.Leptin concentration and DAS28(3)

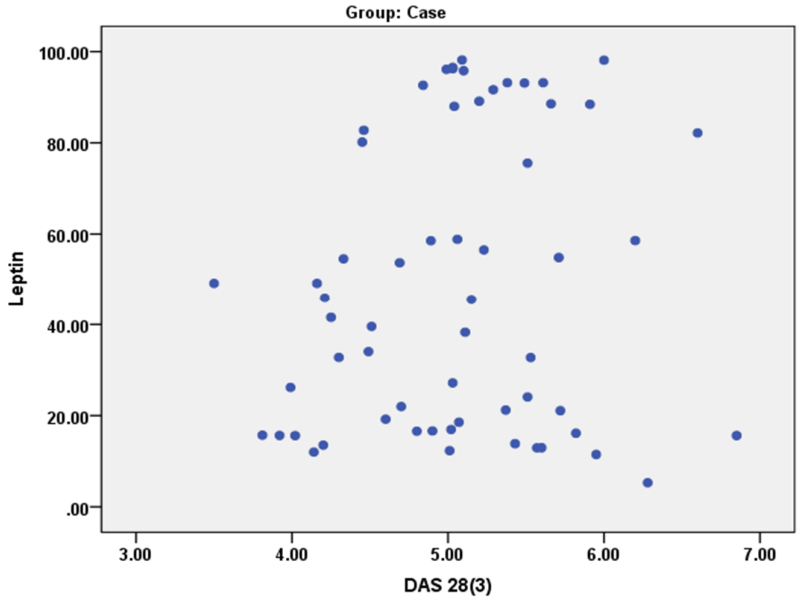


Table 17 Correlation between ESR and Leptin in RA patients

Group: Case		ESR
Leptin	Pearson Correlation	-0.064
	P-Value	0.627
	N	60

Table 17 shows the correlation between leptin and ESR in RA patients. Pearsons correlation coefficient was used to find the association between leptin and ESR. No correlation was observed between leptin and ESR(r = -0.064) and this was not statistically significant (p-value - 0.627).

Table 18 Correlation between leptin and body mass index in RA patients

BMI	Pearson Correlation	0.423
	P-Value	0.001
	N	60

Table 18 shows the correlation between leptin and BMI in RA patients. Pearsons correlation coefficient was used to find the association between leptin

and BMI. A moderate positive correlation was observed between leptin and BMI with r value of 0.423 and this was statistically significant with a p-value of 0.001.

Table 19 Correlation between CRP+VE and Leptin in RA patients

Group: Case		CRP+VE
Leptin	Pearson Correlation	0.44
	P-Value	0.001
	N	51

Table 19 shows the correlation between leptin and CRP+ve RA patients. Pearsons correlation coefficient was used to find the association between leptin and CRP. A good correlation was observed between leptin and CRP with r value of 0.44 and this wast statistically significant with a p-value of 0.001.

Figure 12 Correlation between S.Leptin concentration and CRP

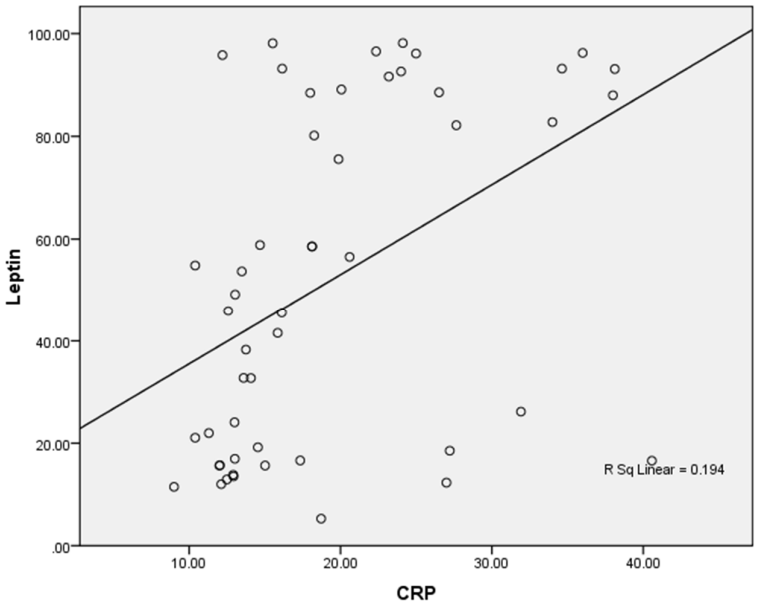


Table 20 shows the charecteristics of study subjects.

Variables	Group	N	Mean	Std. Dev	t-Value	P-Value
Age (yrs)	Case	60	40.75	7.501	0.890	0.376
	Control	30	39.27	7.362		
Age (yrs) : Male	Case	8	43.88	9.433	0.728	0.480
	Control	6	40.00	10.412		
Age (yrs) : Female	Case	52	40.27	7.149	0.686	0.495
	Control	24	39.08	6.678		
Leptin	Case	60	48.7777	32.00198	8.608	<0.001
	Control	30	11.9370	6.11431		
BMI	Case	60	25.8078	4.05917	2.953	0.004
	Control	30	28.3970	3.62473		
CRP	Case	60	17.2872	9.25046	11.043	<0.001
	Control	30	3.8167	1.36257		
ESR	Case	60	34.48	20.001	7.593	<0.001
	Control	30	13.77	4.826		
TC	Case	60	181.22	32.096	1.010	0.315
	Control	30	174.00	31.702		
TGL	Case	60	144.27	59.177	0.658	0.512
	Control	30	152.73	54.112		
HDL	Case	60	50.35	15.026	0.974	0.333
	Control	30	53.60	14.712		
LDL	Case	60	102.0133	37.36382	1.544	0.126
	Control	30	89.8533	30.43509		
Uric acid	Case	60	5.128	1.8616	1.267	0.208
	Control	30	5.650	1.7980		
Urea	Case	60	26.000	5.0321	6.315	<0.001
	Control	30	19.433	3.7571		
Creatinine	Case	60	.970	.1844	1.894	0.062
	Control	30	.893	.1741		

< 0.05 - Significant

<0.01 - Highly Significant

>0.05 - Not Significant

DISCUSSION

Rheumatoid arthritis is a chronic inflammatory autoimmune disease causing functional disability by affecting the articular and extra-articular structures. The disease is characterized by hyperplasia of the synovium, recruitment of inflammatory cells and in its later stages destruction of cartilage and bone. The disease is prevalent worldwide and it occurs by a complex interaction of genetic, environmental and immunological factors.[2] Autoimmunity plays a major role in the pathogenesis of RA.

Studies have demonstrated that several cytokines released by monocytes, macrophages, Th1 and B cells play a significant role in the development of RA. This brings about a break in the immune tolerance leading to autoimmune response. The tissue damage is caused by type III hypersensitivity reaction in AI diseases.[9] The pathogenesis is explained in detail in the Review of literature.

Current hypothesis states that adipokines produced and released by the WAT, plays a major role in the occurrence of chronic proinflammatory state.[3] Changes in the blood levels of systemic and local adipokines or both have been demonstrated in various studies on inflammatory AI diseases. Of all Leptin has been investigated widely as it has been reported to play a pivotal role in the pathogenesis of many AI inflammatory diseases.

Leptin was originally thought as a hormone regulating food intake and energy balance. Later studies revealed the significance of leptin as a neuroendocrine and immunity regulator.[66]

The following features of leptin proves it as a proinflammatory cytokine.

- Structural resemblance to type I cytokine superfamily.
- Similarity of leptin receptor Ob-R to Class I cytokine receptor family.
- Regulation of leptin expression by proinflammatory mediators.
- Elevation of circulating leptin levels in both acute and chronic inflammation.
- Leptin modulation of TH1/TH2 balance, regulating cytokine expression pattern.
- Induction of NOS type II activation in chondrocytes by leptin in synergy with other cytokines.[5]

Moreover when the RA patients were made to fast, an improvement in the clinical parameters of disease activity was observed, with an associated decrease in serum leptin concentration and a shift towards TH2 cytokine production. Therefore from the above facts observed in the studies on RA patients, it was suggested that leptin plays a significant role in the pathogenesis of RA.[136] So this study was conducted to determine the serum leptin status in patients with RA and thereby aid in the future prospective in the control of undesired leptin actions in AI inflammatory disorder.

Sixty RA patients diagnosed by the Rheumatologists at the Rheumatology Out-Patient department of RGGGH, Chennai were selected for this study. The diagnosis was based on the American College of Rheumatology (ACR) Criteria 2010.[137] Both RF+ve and RF-ve patients were included in the study. Thirty age and sex matched controls who had no clinical evidence of RA were selected. Serum Leptin, CRP, uric acid, lipid profile, urea, creatinine, RF, ESR and hemoglobin were determined in all 60 patients and 30 controls. Height, weight and blood pressure were measured for all the patients and controls. BMI was calculated by using "Quetelet Index" and disease activity was assessed by using the DAS 28(3) formula.[138]

In this study population 81.7% (49out of 60) were seen in the age group of 30-50 years of age which agrees with the literature where it is reported that 80% of the patients develop disease between 30- 50 years of age.[1] Also among the cases, female patients 86.7% (52 out of 60) predominated as said in the literature that AI diseases predominate in women.[2]

Among the RA patients, 76.7% (n=46) were RF+ve whereas 23.3% (n=14) were negative for RF. RF positivity is a cardinal feature for RA although it is not specific for RA.

The RA patients exhibited CRP positivity in 85% (n=51) and negativity in 15% (n=9).

ESR was elevated in 80% (n=48) and was normal in 20% (n=12) of the RA patients.

The disease activity score among the RA patients was moderate in 56.7% (n=34) and high in 43.3% (n=26).

BMI was calculated for both cases and controls and they were categorized into normal, overweight and obese groups.

In this study the mean S.Leptin concentration among the cases (n=60) was 48.78±32.00ng/mL which was significantly higher than the control group (n=30) where it was 11.94±6.11ng/mL with a p-value of <0.001 which correlated with previous studies. [139]

The mean S.Leptin concentration in the female patients (n=52) was 49.60±32.03ng/mL which was higher than that of the female controls (n=24)12.52±6.11ng/mL which is statistically significant.[83]

Similarly in the male patients (n=8) S.Leptin concentration is 43.39±33.48ng/mL which was higher when compared to the male controls (n=6) 9.60±6.06ng/mL and it was statistically significant. All these correlate with the literature studies.

Mean S.Leptin concentration in patients with moderate disease activity (n=34) is 46.83±30.92ng/mL and that in high disease activity (n=26) is 51.32±33.81ng/mL. Though the S.Leptin concentration in high disease activity was more than that in the moderate disease activity it was not statistically significant.[140]

S.Leptin concentration in the patients with RA was higher than that of the controls based on BMI. The study showed that patients in the normal, overweight and obese categories showed elevated S.Leptin levels when compared to the same categories in the control group and it was also statistically significant. This reveals that there is association between BMI and leptin as proved in the literature. [67]

The mean S.CRP concentration was higher 17.29±9.25mg/L in the cases (n=60) when compared to that 3.82±1.36mg/L of controls (n=30) and it was statistically significant.

The mean S.Leptin concentration in the CRP+ve patients (n=51) was 52.08±33.12ng/mL and in the CRP-ve patients (n=9) was 30.05±15.22ng/mL and it was statistically significant.

An ANOVA comparison of mean S.Leptin concentration between the 4 groups, RF & CRP positive, only RF positive, only CRP positive, both RF & CRP negative did not reveal any statistically significant difference in serum Leptin concentration.

Further in this study, the DAS showed no correlation with the S.Leptin, where the r value was 0.154 and it was not statistically significant.[140] But there was a good correlation between BMI and S.Leptin levels with a r value = 0.423 and it was statistically significant. [67]A good correlation was also observed

between CRP and leptin which was statistically significant.[141] All the above observations have been reported in previous studies.

So from the above discussion it is obvious that S.Leptin concentration is higher in RA patients when compared to controls and it was statistically significant. There was good correlation between S.Leptin and BMI, however there was no correlation between S.Leptin levels and DAS which was not significant. Moreover a good correlation was observed between leptin and CRP which was statistically significant. Thus the study reveals that leptinemia in RA patients is associated with the pathogenesis of RA.

SUMMARY

Many studies have suggested the significance of Leptin against AI diseases like RA, SLE, Multiple Sclerosis and Psoriasis. Blocking the leptin activity by using leptin mutants and leptin monoclonal antibody have been investigated widely as a strategy for the treatment of RA. The present study was carried out to assess the S.Leptin status in the patients with RA and to correlate it with the disease activity.

A case control study was carried out on 60 RA patients and 30 age and sex matched controls. The S.Leptin levels in RA patients was higher than that of the controls and it was statistically significant.

From the above study we can come to an inference that

- Leptin has a major role in the pathogenesis of RA by its effect on both innate and adaptive immunity.
- As S.Leptin levels reflect the body fat mass, the BMI can be maintained in the normal category and thereby reduce the disease activity.
- Therapeutic targets against leptin signaling can impair the humoral and cellular immune responses on specific target tissues and could be a useful beneficial therapy in the near future.

LIMITATIONS OF THE STUDY

1. The number of male in the study population was low and more number of females were included in the study.
2. Patients aged less than 25years and more than 55 years were not included in the study.
3. The circadian rhythm of leptin could not be taken into consideration in this study.

SCOPE FOR FURTHER STUDIES

1. As the serum leptin concentration is elevated in AI diseases, it can be evaluated in other AI disorders like SLE, Multiple Sclerosis and Psoriasis.
2. As elevated circulating S.Leptin levels can contribute to low grade systemic inflammation, it can be studied in inflammatory disorders like Coronary Artery Atherosclerosis, Type II Diabetes Mellitus or Osteoarthritis.
3. S.Leptin concentration can be studied in relation to other inflammatory cytokines in RA patients.
4. Relationship between S.Leptin levels and radiographic joint damage in RA patients can be studied in future.
5. Studies on therapeutic trials with leptin receptor antagonists can be tried.

BIBLIOGRAPHY

1. KELLEY'S Textbook of Rheumatology; Ninth edition; Part 9; Chapter 69; pg1059.
2. KELLEY'S Textbook of Rheumatology; Ninth edition; Part 9; Chapter 69; pg1063.
3. Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochem Soc Trans 2005;33(Pt 5):1078–81.
4. Baumann H, Morella KK, White DW, Dembski M, Bailon PS, Kim H, Lai CF, Tartaglia LA: The full-length leptin receptor has signalling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci USA* 1996, 93:8374-8378.
5. Otero M, Gomez Reino JJ, Gualillo O. Synergistic induction of nitricoxide synthase type II: in vitro effect of leptin and interferon-gamma in human chondrocytes and ATDC5 chondrogenic cells. Arthritis Rheum 2003;48:404–9.
6. Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987American College of Rheumatology criteria: a systematic review. Semin Arthritis Rheum 2006;36:182-8.
7. KELLEY'S Textbook of Rheumatology; Ninth edition; Part 9; Chapter 70; pg1109.
8. Malaviya AN, Kapoor SK, Singh RR, Kumar A, Pande I. Prevalence of rheumatoid arthritis in the adult Indian population. *Rheumatol Int.* 1993;13(4):131-34.
9. Clinical immunology principles & practice 4 th edition chapter 51 Rheumatoid arthritis Andrew P.Cope
10. Karlson EW, Mandl LA, Hankinson SE, Grodstein F. Do breast-feeding and other reproductive factors influence future risk of rheumatoid

arthritis? Results from the Nurses' Health Study. Arthritis Rheum2004;50:3458-67.

11. Pikwer M, Bergstrom U, Nilsson JA, Jacobsson L, Turesson C. Early menopause is an independent predictor of rheumatoid arthritis. Ann Rheum Dis2012;71:378-81.
12. Spector TD, Hochberg MC. The protective effect of the oral contraceptive pill on rheumatoid arthritis: an overview of the analytic epidemiological studies using meta-analysis. J Clin Epidemiol 1990;43:1221-30.
13. Jacobsson LT, Jacobsson ME, Askling J, Knowler WC. Perinatal characteristics and risk of rheumatoid arthritis. BMJ 2003;326:1068-9.
14. Lahiri M, Morgan C, Symmons DP, Bruce IN. Modifiable risk factors for RA: prevention, better than cure? Rheumatology (Oxford) 2012;51:499-512.
15. Hutchinson D, Moots R. Cigarette smoking and severity of rheumatoid arthritis. Rheumatology (Oxford) 2001;40:1426-7.
16. Klareskog L, Stolt P, Lundberg K, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 2006;54:38-46.
17. Pedersen M, Jacobsen S, Klarlund M, et al. Environmental risk factors differ between rheumatoid arthritis with and without auto-antibodies against cyclic citrullinated peptides. Arthritis Res Ther 2006;8:R133.
18. Voigt LF, Koepsell TD, Nelson JL, Dugowson CE, Daling JR. Smoking, obesity, alcohol consumption, and the risk of rheumatoid arthritis. Epidemiology 1994;5:525-32.
19. Benito-Garcia E, Feskanich D, Hu FB, Mandl LA, KarlsonEW. Protein, iron, and meat consumption and risk forrheumatoid arthritis: a prospective cohort study. Arthritis Res Ther 2007;9:R16.

20. Jick SS, Choi H, Li L, McInnes IB, Sattar N. Hyperlipidaemia, statin use and the risk of developing rheumatoid arthritis. Ann Rheum Dis 2009;68:546-51.

21. Kouri T, Petersen J, Rhodes G, et al. Antibodies to synthetic peptides from Epstein-Barr nuclear antigen-1in sera of patients with early rheumatoid arthritis and in preillness sera. J Rheumatol 1990;17:1442-9.

22. Hajeer AH, MacGregor AJ, Rigby AS, et al. Influence of previous exposure to human parvovirus B19 infection in explaining susceptibility to rheumatoid arthritis: an analysis of disease discordant twin pairs. Ann Rheum Dis 1994;53:137-9.

23. Stolt P, Kallberg H, Lundberg I, et al. Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. Ann Rheum Dis 2005;64:582-6.

24. Hart JE, Laden F, Puett RC, Costenbader KC, Karlson EW. Exposure to traffic pollution and increased risk of rheumatoid arthritis. Environ Health Perspect 2009;117:1065-9.

25. Firestein GS, Zvaifler NJ: How important are T cells in chronic rheumatoid synovitis?: II. T cell-independent mechanisms from beginning to end, *Arthritis Rheum* 46:298, 2002.

26. Weyand CM, Hicok KC, Conn DL, Goronzy JJ: The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis, *Ann Intern Med* 117:801, 1992.

27. Van Der Woude D, Lie BA, Lundström E, et al: Protection against anti citrullinated protein antibody-positive rheumatoid arthritis is predominantly associated with HLA-DRB1*1301: a meta-analysis of HLA-DRB1 associations with anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis in four European populations, *Arthritis Rheum* 62:1236, 2010.

28. Rak JM, Maestroni L, Balandraud N, et al: Transfer of the shared epitope through microchimerism in women with rheumatoid arthritis, *Arthritis Rheum* 60:73, 2009.

29. Kelly's textbook of Rheumatology,ninth edition ,volume 2 page 1062.

30. Suzuki A, Yamada R, Chang X, et al: Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis, *Nat Genet* 34:395, 2003.

31. Begovich AB, Carlton VE, Honigberg LA, et al: A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis, *Am J Hum Genet* 75:330, 2004.

32. Stahl EA, Raychaudhuri S, Remmers EF, et al: Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci, *Nat Genet* 42:508–514, 2010.

33. Nelson JL, Hughes KA, Smith AG, et al: Maternal-fetal disparity in HLA class II alloantigens and the pregnancy-induced amelioration of rheumatoid arthritis, *N Engl J Med* 329:466, 1993.

34. Karouzakis E, Gay RE, Michel BA, et al: DNA hypomethylation in rheumatoid arthritis synovial fibroblasts, *Arthritis Rheum* 60:3613, 2009.

35. Choe JY, Crain B, Wu SR, Corr M: Interleukin 1 receptor dependence of serum transferred arthritis can be circumvented by toll-like receptor 4 signaling, *J Exp Med* 197:537, 2003.

36. Kelly's textbook of Rheumatology,ninth edition ,volume 2 page 1065.

37. Kelly's textbook of Rheumatology,ninth edition ,volume 2 page 1066

38. Nielen MM, van Schaardenburg D, Reesink HW, et al: Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors, *Arthritis Rheum* 50:38, 2004.

39. Liang KP, Maradit Kremers H, Crowson CS, et al: Autoantibodies and the risk of cardiovascular events, *J Rheumatol* 36(11):2462–2468, 2009.

40. De Rycke L, Nicholas AP, Cantaert T, et al: Synovial intracellular citrullinated proteins colocalizing with peptidyl arginine deiminase as pathophysiologically relevant antigenic determinants of rheumatoid arthritis-specific humoral autoimmunity, *Arthritis Rheum* 52:2323, 2005.

41. Haisma EM, Levarht EW, van der Woude D, et al: Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways, *Arthritis Rheum* 60:1923, 2009.

42. Kelly's textbook of Rheumatology,ninth edition ,volume 2 page 1069.

43. Mandik-Nayak L, Allen PM: Initiation of an autoimmune response: insights from a transgenic model of rheumatoid arthritis, *Immunol Res* 32:5, 2005.

44. Nell VP, Machold KP, Stamm TA, et al: Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis, *Ann Rheum Dis* 64:1731, 2005.

45. Unemori EN, Bair MJ, Bauer EA, Amento EP: Stromelysin expression regulates collagenase activation in human fibroblasts. Dissociable control of two metalloproteinases by interferon-gamma, *J Biol Chem* 266:23477, 1991.

46. Raza K, Falciani F, Curnow SJ, et al: Early rheumatoid arthritis is characterized by a distinct and transient synovial fluid cytokine profile of T cell and stromal cell origin, *Arthritis Res Ther* 7:R784, 2005.

47. Chabaud M, Durand JM, Buchs N, et al: Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium, *Arthritis Rheum* 43:963, 1999.

48. Firestein GS, Alvaro-Gracia JM, Maki R: Quantitative analysis of cytokine gene expression in rheumatoid arthritis, *J Immunol* 144:3347, 1990.

49. Joosten LA, van De Loo FA, Lubberts E, et al: An IFN-gamma-independent proinflammatory role of IL-18 in murine streptococcal cell wall arthritis, *J Immunol* 165:6553, 2000.

50. Lipsky PE, van der Heijde DM, St Clair EW, et al: Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group, *N Engl J Med* 343:1594, 2000.

51. Joosten LA, Netea MG, Kim SH, et al: IL-32, a proinflammatory cytokine in rheumatoid arthritis, *Proc Natl Acad Sci U S A* 103:3298, 2006.

52. Xu WD, Firestein GS, Taetle R, et al: Cytokines in chronic inflammatory arthritis. II. Granulocyte-macrophage colony-stimulating factor in rheumatoid synovial effusions, *J Clin Invest* 83:876, 1989.

53. Koch AE, Kunkel SL, Burrows JC, et al: Synovial tissue macrophage as a source of the chemotactic cytokine IL-8, *J Immunol* 147:2187, 1991.

54. Koch AE, Kunkel SL, Harlow LA, et al: Epithelial neutrophil activating peptide-78: a novel chemotactic cytokine for neutrophils in arthritis, *J Clin Invest* 94:1012, 1994.

55. Schmutz C, Hulme A, Burman A, et al: Chemokine receptors in the rheumatoid synovium: upregulation of CXCR5, *Arthritis Res Ther* 7:R217, 2005.

56. Kelly's textbook of Rheumatology,ninth edition ,volume 2 page 1092.

57. Harrison's Principles of Internal Medicine; Volume 2, page 1883.

58. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics; Fifth Edition, page 789.

59. Lehninger Principles of Biochemistry, Fifth edition, page 931.

60. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372(6505): 425–32. [PubMed: 7984236]

61. Friedman JM: Obesity in the new millenium. *Nature* 2000; 404:632-634.

62. Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature (Lond.) 372:425̃432.

63. Isse N, Ogawa Y, Tamura N, Masuzaki H, Mori K, et al. 1995. Structural organization and chromosomal assignment of the human obese gene. *J. Biol. Chem.*270:27728–33

64. Gong DW, Bi S, Pratley RE, Weintraub BD. 1996. Genomic structure and promoter analysis of the human obese gene. *J. Biol. Chem.* 271:3971–74.

65. Grasso P, Leinung MC, Inher SP, Lee DW. 1997. In vitro effects of leptin related synthetic peptides on body weight and food intake in female ob/ob mice: localization of leptin activity to domains between amino acid residues 106–140. *Endocrinology* 138:1413–18

66. Madej T, Boguski MS, Bryant SH. 1995. Threading analysis suggests that the obese gene product may be a helical cytokine. *FEBS Lett.* 373:13–18

67. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, et al. 1996. Serum immunoreactive leptin concentrations in normal weight and obese humans. *N. Engl. J. Med.* 334:292–95

68. Montague CT, Farooqui S, Whitehead JP, Soos MA, Rau H, et al. 1997. Congenital leptin deficiency is associated with severe early onset obesity in humans. *Nature* 387:903–8

69. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. 1998. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat. Genet.* 18:213–15

70. Ozata M, Ozdemir IC, Licinio J: Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. *J Clin Endocrinol Metab* 1999, 84:3686-3695.

71. Lonnqvist F, Arner P, Nordfors, Schalling W. 1995. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nat. Med.* 1:950– 53.

72. Saladin R, Devos P, Guerre-Millo M, Leturge A, Girard J, et al. 1995. Transient increase in obese gene expression after food intake or insulin administration. *Nature* 377:527–29

73. Licinio J, Mantzoros C, Negrao AB, Cizza G, Wong ML, et al. 1997. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. *Nat. Med*. 3:575–79

74. Mantzoros C, Flier JS, Rogol AD. 1997. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. *J. Clin. Endocrinol. Metab*. 82:1066–70

75. Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, et al. 1996. Effects of gender, body composition and menopause on plasma concentration of leptin. *J. Clin. Endocrinol. Metab* 81: 3424–27

76. Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, et al. 1997. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, fat mass, gender, pubertal stage, and testosterone. *J. Clin. Endocrinol. Metab*. 82: 2904–10

77. Pinkney JH, Goodrick SJ, Katz J, Johnson AB, Lightman SL, Coppack SW, et al. 1998. Leptin and the pituitary-thyroid axis: a comparative study

in lean, obese, hypothyroid and hyperthyroid subjects. *Clin. Endocrinol.* 49:583–88

78. Bornstein SR, Licinio J, Tauchnitz R, Engelmann L, Negrao AB, Chrousos GP. 1998. Plasma leptin levels are increased in survivors of acute sepsis: associated loss of diurnal rhythm in cortisol and leptin secretion. *J. Clin. Endocrinol. Metab.*83:280–83

79. Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, et al. 1997. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J. Exp. Med.* 185:171–75

80. Trayhurn P, Duncan JS, Rayner DV. 1996. Acute cold-induced suppression of Ob (obese) gene expression in white adipose tissue of mice: mediation by the sympathetic nervous system. *Biochem. J.* 311:729–33

81. Danahoo WT, Jensen DR, Yost TJ, Eckel RH. 1997. Isoproterenol and somatostatin decrease plasma leptin in humans: a novel mechanism regulating leptin secretion. *J. Clin. Endocrinol. Metab.* 82: 4139–43

82. Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, et al. 1997. Nonadipose production of leptin: leptinas a novel placenta-derived hormone in humans. *Nat. Med.* 3:1029–33

83. Bado A, Levasseur S, Attoub S, Kermogant S, Laigneau JP, et al. 1998. The stomach is a source of leptin. *Nature* 394:790–93

84. Shekhawat PS, Garland JS, Shivpuri C, Mick GJ, Sasidharan A, et al. 1998. Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids. *Pediatr. Res.* 43:338–43

85. Casabiell X, Pineiro V, Tome MA, Peino R, Dieguez C, Casanueva FF. 1997. Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. *J. Clin. Endocrinol. Metab*. 82: 4270–73

86. Wang J, Liu R, Hawkins M, Barzalai N, Rossetti L. 1998. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* 393:684–88

87. Tartaglia L, Dembski M, Weng X, Deng N, Culpper J, et al. 1995. Identification and expression cloning of a leptin receptor. *Cell* 83:1263–71

88. Lee GH, Proenca R, Montez JM, Carroll K, Darvishzadeh JG, et al. 1996. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379:632–35

89. Bjorbaek C, Uotani S, da Silva B, Flier JS. 1997. Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J. Biol. Chem.* 272:32686–95

90. Faggioni R, Feingold KR, Grunfeld C. Leptin regulation of the immune response and the immunodeficiency of malnutrition. FASEB J 2001; 15:2565–71.

91. Smith JT, Waddell BJ. Leptin receptor expression in the rat placenta: changes in ob-ra, ob-rb, and ob-re with gestational age and suppression by glucocorticoids. Biol Reprod 2002; 67:1204–10.

92. Elmquist JK, Ahima RS, Elias CS, Flier JS, Saper CB. 1998. Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. *Proc. Natl. Acad. Sci. USA* 741–48

93. Bjorbaek C, Elmquist JK, Michl P, Ahima RS, van Bueren A, et al. 1998. Expression of leptin receptor isoforms in brain microvessels. *Endocrinology* 139:3485–91

94. Stryer Textbook of Biochemistry; Seventh Edition, page 826.

95. William's Textbook of Endocrinolgy; Twelfth Edition, page 1612.

96. Lehninger Principles of Biochemistry; Fifth Edition, page 933.

97. Liu Q, Bai C Chen F et al. Uncoupling protein-3 a muscle specific gene upregulated by leptin in ob/ob mice. Gene 1998, 207:1-7.

98. Rossetti L, Massillon D, Barzilai N, Vuguin P, Chen W, et al. 1997. Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. *J. Biol. Chem.* 272:27758–63

99. Kamohara S, Burcelin R, Halaas JL, Friedman J, Charron M. 1997. Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* 389:374–77

100. Cohen SM, Werrman J, Tota M. 1998. 13C NMR study of the effects of leptin treatment on kinetics of hepatic intermediary metabolism. *Proc. Natl. Acad. Sci. USA* 95:7385–90

101. Siegrist-Kaiser C, Pauli V, Juge-Aubry C, Boss O, Pernin A, et al. 1997. Direct effect of leptin on brown and white adipose tissue. *J. Clin. Invest.* 100:2858–64

102. Bai Y, Zhang S, Kim KS, Lee JK, Kim KH. Obese gene expression alters the ability of 30AS preadipocytes to respond to lipogenic hormones.*J Biol Chem* 1996; 271:13939-42.

103. Lehninger Principles of Biochemistry; Fifth Edition, page 934

104. Carro E, Senaris R, Considine RV, et al: Regulation of in vivo growth hormone secretion by leptin. *Endocrinology* 1997; 138:2203-2206.

105. Okada K, Sugihara H, Minami S, Wakabayashi I: Effect of parenteral administration of selected nutrients and central injection of gamma-globulin from antiserum to neuropeptide Y on growth hormone secretory pattern in food-deprived rats. *Neuroendocrinology* 1993; 57:678-686.

106. al-Shoumer KA, Anyaoku V, Richmond W, Johnston DG: Elevated leptin concentrations in growth hormone–deficient hypopituitary adults. *Clin Endocrinol (Oxf)* 1997; 47:153-159.

107. Vettor R: The metabolic actions of thyroid hormone and leptin: a mandatory interplay or not?. *Diabetologia* 2005; 48:621-623.

108. Iglesias P, Alvarez Fidalgo P, Codoceo R, et al: Serum concentrations of adipocytokines in patients with hyperthyroidism and hypothyroidism before and after control of thyroid function. *Clin Endocrinol (Oxf)* 2003; 59:621-629.

109. Flier JS. 1998. Clinical review 94: What's in a name? In search of leptin's physiologic role. *J. Clin. Endocrinol. Metab*. 83:1407–13

110. Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS. 1997. Leptin inhibition of the hypothalamic-pituitary- adrenal axis in response to stress. *Endocrinology* 138:3859–63

111. Moschos S, Chan JI, Mantzoros CS. Leptin and Reproduction: a review. Fertil Steril./2002; 77: 433-444.

112. Cunningham MJ, Clifton DK, Steiner RA. Leptin's actions on the reproductive axis: perspectives and mechanisms. Boil Reprod. 1999; 60: 216-222.

113. Wabitsch M, Ballauf A, Holl R, et al. Serum leptin, gonadotropin and testosterone concentrations in male patients with anorexia nervosa during weight gain. J Clin Endocrinol Metab. 2001; 86: 2982-2988.

114. Donatto JJ, Cravo RM, Frazao, et al. Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not reguice signalling in kiss the neurons. J Clin Invest. 2001; 121: 355-368.

115. Gimble, J. M., C. E. Robinson, X. Wu, and K. A. Kelly. 1996. The function of adipocytes in the bone marrow stroma: An update. Bone 19:42̃1428.

116. Ghilardi, N., and R. C. Skoda. 1997. The leptin receptor activates Janus Kinase 2 and signals for proliferation in a factor-dependent cell line. Mol. Endocrinol. 11:39̃3399.

117. Stryer Textbook of Biochemistry; Seventh edition, page 827.

118. Spiegelman BM, Flier JS. 1996. Adipogenesis and obesity: rounding out the big picture. *Cell* 87:377–89

119. Carpenter LR, Farruggela TJ, Symes A, Karow ML, Yancopoulos GD, et al. 1998. Enhancing leptin response by preventing SH2-containing phosphatase 2 interaction with ob receptor. *Proc. Natl. Acad. Sci. USA* 95:6061–66

120. Zarkesh-Esfahani H, Pockley G, Metcalfe RA et al. High-dose leptin activates human leukocytes via receptor expression on monocytes. J Immunol 2001;167:4593- 9.

121. Tian Z, Sun R, Wei H, Gao B. Impaired natural killer (NK) cell activity in leptin receptor deficient mice: leptin as a critical regulator in NK cell development and activation. Biochem Biophys Res Commun 2002;298:297–302.

122. Ruter J, Hoffmann T, Demuth HU, Moschansky P, Klapp BF, Hildebrandt M. Evidence for an interaction between leptin, T cell costimulatory antigens CD28, CTLA-4 and CD26 (dipeptidyl peptidase IV) in BCG-induced immune responses of leptin- and leptin receptor-deficient mice. Biol Chem 2004;385:537–41.

123. Farooqi IS, Matarese G, Lord GM et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest 2002;110:1093–103.

124. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation induced immunosuppression. Nature 1998;394:897–901.

125. Faggioni R, Feingold KR, Grunfeld C. Leptin regulation on the immune response and the immunodeficiency of malnutrition. FASEB J 2001;14:2565–71.

126. Faggioni R, Fantuzzi G, Gabay C, Moser A, Dinarello CA, Feingold KR, Grunfeld C. Leptin deficiency enhances sensitivity to endotoxin-induced lethality. Am J Physiol 1999;276:136–42.

127. Williams L, Bradley L, Smith A, Foxwell B. Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. J Immunol 2004;172:567–76.

128. Matarese G, Carrieri PB, La Cava A. Leptin increase in multiple sclerosis associates with reduced number of CD4(þ)CD25þ regulatory T cells. Proc Natl Acad Sci 2005;102:5150–5.

129. Banks AS, Davis SM, Bates SH, Myers MG Jr. Activation of downstream signals by the long form of the leptin receptor. J Biol Chem 2000; 275:14563–72.

130. Van den Brink GR, O'Toole T, Hardwick JC *et al*. Leptin signalling in human peripheral blood mononuclear cells, activation of p38 and p42/44 mitogen-activated protein (MAP) kinase and p70 S6 kinase. Mol Cell Biol Res Commun 2000; 4:144–50.

131. Lam QL, Lu L. Role of leptin in immunity. Cell Mol Immunol 2007; 4:1–13.

132. Elbatarny HS, Maurice DH. Leptin-mediated activation of human platelets: involvement of a leptin receptor and phosphodiesterase 3A-containing cellular signaling complex. Am J Physiol Endocrinol Metab 2005; 289:E695–702.

133. Morris DL, Rui L. Recent advances in understanding leptin signaling and leptin resistance. Am J Physiol Endocrinol Metab 2009; 297:E1247–59.

134. Muraoka S, Kusunoki N, Takahashi H, Tsuchiya K, Kawai S. Leptin stimulates interleukin-6 production via janus kinase 2/signal transducer and activator of transcription 3 in rheumatoid synovial fibroblasts. Clin Exp Rheumatol 2013; 31:589–95.

135. Harle P, Pongratz G, Weidler C, Buttner R, Scholmerich J, Straub RH. Possible role of leptin in hypoandrogenicity in patients with systemic lupus erythematosus and rheumatoid arthritis. Ann Rheum Dis 2004;63:809–16.

136. Fraser DA, Thoen J, Reseland JE, Forre O, Kjeldsen-Kragh J. Decreased CD4+ lymphocyte activation and increased interleukin- 4 production in peripheral blood of rheumatoid arthritis patients after acute starvation. Clin Rheumatol 1999;18:394–401.

137. Harald E .Vonkeman, Mart A.F.J.Van de Laar- the new EULAR/ ACR diagnostic criteria for Rheumatoid arthritis- curr opin Rheumatol,2013;25(3):354-359.

138. Jaap Fransen, Gerold Stucki, and Piet L. C. M. van Riel,Rheumatoid Arthritis Measures Disease Activity Score (DAS), Disease Activity Score-28 (DAS28), Rapid Assessment of Disease Activity in Rheumatology (RADAR), and Rheumatoid Arthritis Disease Activity Index (RADAI) Arthritis & Rheumatism (Arthritis Care & Research) Vol. 49, No. 5S, October 15, 2003, pp S214–S224

139. Salazar-Paramo M, Gonzalez-Ortiz M, Gonzalez-Lopez L, Sa´ nchez-Ortiz A, Valera-Gonza´ lez I, Martı´nez-Abundis E, et al. Serum leptin levels in patients with rheumatoid arthritis. J Clin Rheumatol 2001;7:57–9.

140. Anders HJ, Rihl M, Heufelder A, Loch O, Schattenkirchner M. Leptin serum levels are not correlated with disease activity in patients with rheumatoid arthritis. Metabolism 1999;48:745–8.

141. Takumi, Natsuko K, Nahoko Tanaka et al. Elevated serum levels of Resistin, Leptin & adiponectin are associated with C-reactive protein and also other clinical condition in Rheumatoid arthritis. Intern Med 50:269-275; 2011.

Annexures

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To

Dr.S.Michael Rajam Geetha
II Year PG in MD (Bio-Chemistry)
Institute of Bio-Chemistry
Madras Medical College
Chennai 600 003

Dear Dr.S.Michael Rajam Geetha,

The Institutional Ethics Committee has considered your request and approved your study titled **"SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS "** NO.12012015.

The following members of Ethics Committee were present in the meeting hold on 20.01.2015 conducted at Madras Medical College, Chennai 3.

1. Dr.C.Rajendran, MD	:Chairperson
2. Dr.R.Vimala,MD.,Dean,MMC,Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi,MD.,Vice Principal,MMC,Ch-3	: Member Secretary
4. Prof.R.Nandhini,MD.,Inst.of Pharmacology,MMC	: Member
5. Prof.P.Ragumani, MS., Professor, Inst.of Surgery,MMC	: Member
6. Prof.K.Ramadevi, Director , Inst.of Bio-Chem.MMC	: Member
7. Prof.Saraswathy,MD.,Director,Pathology, MMC	: Member
8. Prof.Md.Ali, MD., DM.,Prof.&HOD of Medl.GE,MD.MMC	: Member
9. Thiru S.Rameshkumar	: Lay Person
10.Thiru S.Govindasamy, BA., BL.,	: Lawyer
11.Tmt.Arnold Saulina, MA., MSW.,	: Social Scientist

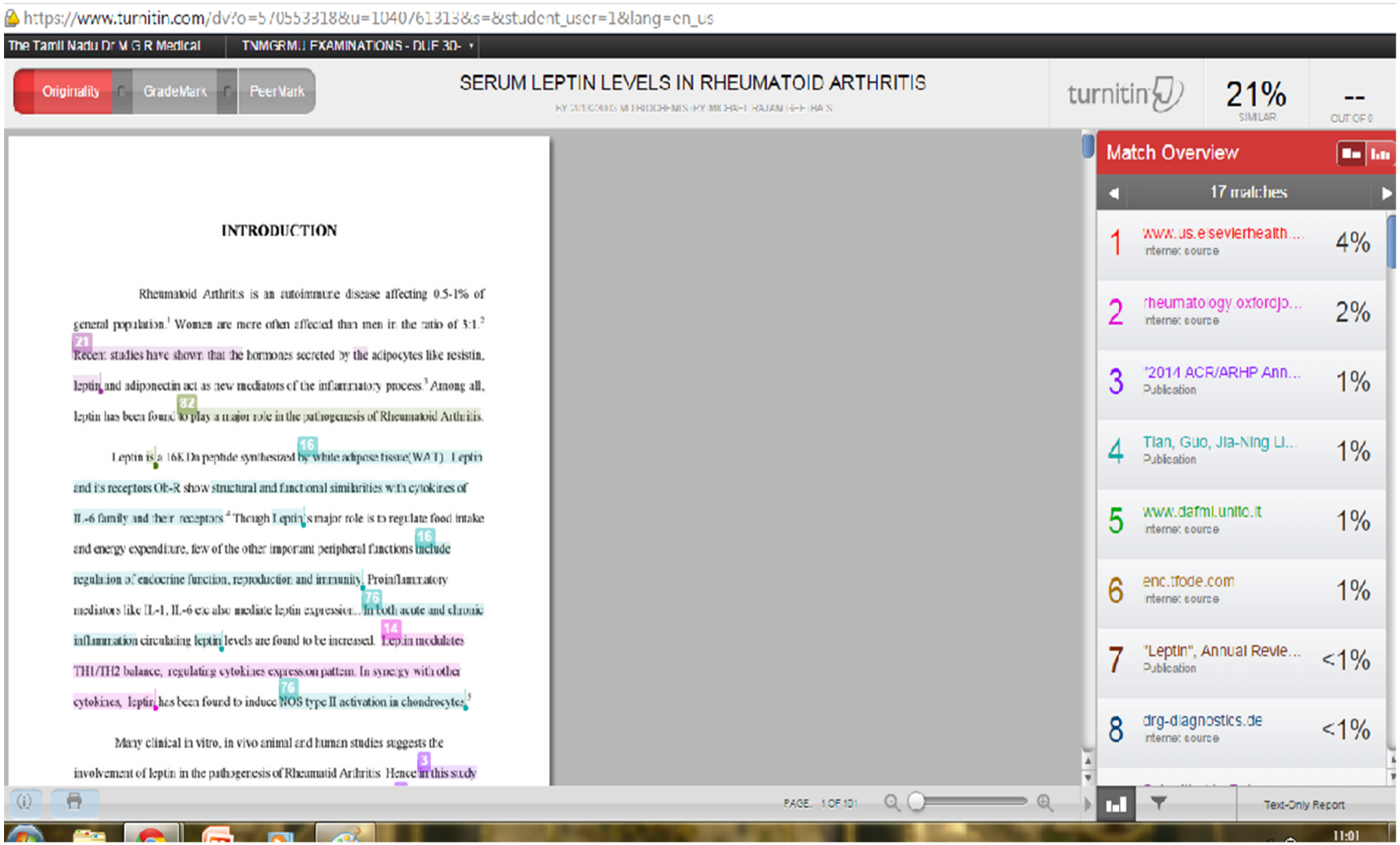
We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary - Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

Sys 2


https://www.turnitin.com/dv?o=570553318&u=1040761313&s=&student_user=1&lang=en_us
The Tamil Nadu Dr M G R Medical
TNMGRMU EXAMINATIONS - DUE 30-
Originality
GradeMark
PeerMark
SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS
turnitin
21%
SIMILAR
--
OUT OF 0
INTRODUCTION
Rheumatoid Arthritis is an autoimmune disease affecting 0.5-1% of general population.[1] Women are more often affected than men in the ratio of 3:1.[2] Recent studies have shown that the hormones secreted by the adipocytes like resistin, leptin and adiponectin act as new mediators of the inflammatory process.[3] Among all, leptin has been found to play a major role in the pathogenesis of Rheumatoid Arthritis.
Leptin is a 16K Da peptide synthesized by white adipose tissue(WAT). Leptin and its receptors Ob-R show structural and functional similarities with cytokines of IL-6 family and their receptors.[4] Though Leptin's major role is to regulate food intake and energy expenditure, few of the other important peripheral functions include regulation of endocrine function, reproduction and immunity. Proinflammatory mediators like IL-1, IL-6 etc also mediate leptin expression. In both acute and chronic inflammation circulating leptin levels are found to be increased. Leptin modulates TH1/TH2 balance, regulating cytokines expression pattern. In synergy with other cytokines, leptin has been found to induce NOS type II activation in chondrocytes.[5]
Many clinical in vitro, in vivo animal and human studies suggests the involvement of leptin in the pathogenesis of Rheumatid Arthritis. Hence in this study
PAGE 1 OF 101
Match Overview
17 matches
1 www.us.elsevierhealth... 4%
Internet source
2 rheumatology.oxfordjo... 2%
Internet source
3 "2011 ACR/ARHP Ann... 1%
Publication
4 Tian, Guo, Jia-Ning Li... 1%
Publication
5 www.dafml.unito.it 1%
Internet source
6 enc.tfode.com 1%
Internet source
7 "Leptin", Annual Revie... <1%
Publication
8 drg-diagnostics.de <1%
Internet source
Text-Only Report
11:01

INFORMATION SHEET

Title : SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS

Investigator : Dr.S.Michael Rajam Geetha,
Postgraduate,
Institute of Biochemistry,
Madras Medical College,
Chennai-600003.

Guide : Dr.V.K.Ramadesikan,
Professor,
Institute of Biochemistry,
Madras Medical College,
Chennai-600003.

Rheumatoid arthritis affects 1-2% of the general population.It affects women 3 times more than men. It causes symmetrical synovitis, polyarthritis, progressive joint damage, pain, disfigurement and disability. It also affects other systems like cardiovascular system, respiratory system, excretory system etc. Recent studies have shown that leptin, an adipocyte hormone plays an important role in the pathogenesis of rheumatoid arthritis.By measuring the levels of leptin in rheumatoid arthritis patients we can know the significance of leptin in the causation of rheumatoid arthritis and thereby aid in the future diagnostic and therapeutic interventions.

Hence I am doing this study titled “Role of leptin in rheumatoid arthritis” at Rajiv Gandhi Govt. General Hospital, Chennai.To do this study I need to collect 5ml of blood from 60 rheumatoid arthritis patients and 30 apparently healthy individuals. While collecting the blood there will be no side effects.

Your identity will be kept confidential throughout the study and also during publication or presentation ofthe study findings in any clinical forum or journals. Participation in this study is purely voluntary. You can withdraw from this study at any time. Your decision will not result in any loss of benefits to which you are entitled. The results of the study will be intimated to you. If you have willingness to participate in this study, kindly sign in this information sheet and the consent form.

Signature of the investigator

Signature of the participant

Thumb impression

Place:

Date:

PATIENT CONSENT FORM

Title of the study **:" SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS."**

Name :
Age :
Sex :
:

Date :
OP No:
Project Patient No

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I hereby give my consent to be included as a participant in "**SERUM LEPTIN LEVELS IN RHEUMATOID ARTHRITIS."**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have informed the investigator of all the treatments I am taking or have taken for the past --------- months/years including any native (alternative) treatment.
6. I have been informed about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past _____ month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.
11. I hereby give permission to the investigators to release the information obtained from me as a result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
12. I have understood that my identity will be kept confidential even if my data are publicly presented.
13. I have had my questions answered to my satisfaction.
14. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For participants:

Name and signature / thumb impression of the participant (or legal representative if participant in competent/For age 10-17 yrs-Name& signature of the parent/guardian.)

Name _____ Signature with date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature with date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature with Date_____

ஆராய்ச்சி தகவல் தாள்

தலைப்பு:

முடக்குவாத நோயாளிகளின் இரத்தத்தில் லெப்டின் நிலை.

ஆராய்ச்சியாளர் : **மரு. செ.மைக்கேல் ராஜம் கீதா,**
பட்ட மேற்படிப்பு மருத்துவ மாணவி,
உயிர்வேதியியல் உயர்நிலைத்துறை,
சென்னை மருத்துவக் கல்லூரி மருத்துவமனை,
சென்னை - 600003.

ஆராய்ச்சி மேற்பார்வையாளர் : **மரு. வி.கே. இராமதேசிகன்,**
துணை பேராசிரியர்,
உயிர்வேதியியல் உயர்நிலைத்துறை,
சென்னை மருத்துவக் கல்லூரி மருத்துவமனை,
சென்னை - 600003.

பொதுமக்களில் 1-2% வரை முடக்குவாதம் தாக்குகின்றது. ஆண்களைவிட பெண்கள் 3 மடங்கு அதிகமாக பாதிப்புக்குள்ளாகின்றனர். இந்த நோயினால் மூட்டுச்சவ்வு மற்றும் மூட்டுக்கள் அதிகமாக பாதிக்கப்படுவதால் நோயாளிகளுக்கு மிகுந்த வேதனையும், உடல் அங்கவீளமும் ஏற்படுகின்றது. மேலும் மற்ற முக்கிய உறுப்புகளான இதயம், நுரையீரல், சிறுநீரகங்கள் பாதிக்கப்படுகின்றது. கொழுப்பு செலில் இருந்து வரும் லெப்டின் என்றும் வேதிப்பொருள் (இயக்குநீர்) முடக்குவாதம் உண்டாக்குவதில் முக்கிய பங்கு வகிப்பதாக தற்போது கண்டறியப்பட்டுள்ளது. எனவே இதைப் பற்றி அறிதல் தற்போதைய நோயின் கண்டறியும் முறையிலும், சிகிச்சை முறையிலும் சில மாற்றங்களை ஏற்படுத்தி நோயாளிகளுக்கு பயன் அளிக்க வாய்ப்புள்ளது.

எனவே சென்னை இராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் முடக்குவாத நோயாளிகளின் இரத்தத்தில் லெப்டின் அளவினை கண்டறியும் ஆராய்ச்சியில் ஈடுபட்டுள்ளேன்.

இதற்கு 60 முடக்குவாத நோயாளிகளிடமும், அவர்களின் பாலினம் மற்றும் வயதிற்கு ஏற்றார் போலுள்ள, 30 ஆரோக்கியமான (முடக்குவாதம் மற்றும் வேறு எந்த நோயில்லாத) நபர்களிடமும் 5 மி.லி. இரத்தம் எடுத்து ஆராய்ச்சிக்கு உட்படுத்த உள்ளேன்.

தங்களிடமிருந்து ஊசியின் மூலம் 5 மி.லி. இரத்தம் எடுப்பதனால் எந்தவிதமான பக்க விளைவுகளும் ஏற்படாது என உறுதி அளிக்கின்றேன்.

தாங்கள் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இதில் பங்கு பெறுவதினால் நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு உள்ளாகாது என்பதையும் கூடுதல் செலவீனம் ஏற்படாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயர் மற்றும் அடையாளங்கள் வெளியிடப்படாது என்பதை தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களின் விருப்பத்தின் பேரில் தான் இருக்கிறது. எந்நேரமும் இதிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியின் முடிவுகளை தங்களுக்கு அறிவிப்போம் என்பதை தெரிவித்துக் கொள்கிறோம்.

நீங்கள் இந்த ஆராய்ச்சியில் பங்குபெற விருப்பம் இருப்பின் இந்த தகவல்தாள் மற்றும் ஆராய்ச்சி ஒப்புதல் படிவத்திலும் கையொப்பம் இடுமாறு கேட்டுக் கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம்

பங்கேற்பாளர் கையொப்பம்
/இடது கைவிரல் ரேகை

இடம் :

தேதி :

நோயாளியின் ஒப்புதல் படிவம்

தலைப்பு:

முடக்குவாத நோயாளிகளின் இரத்தத்தில் லெப்டின் நிலை.

பங்கேற்பாளர் பெயர் : புற / உள் நோயாளி எண்:

வயது : பால் : கைபேசி/தொலைபேசி எண் :

முகவரி : ஆராய்ச்சி சேர்க்கை எண் :

.....ஆகிய நான் மரு. செ.மைக்கேல் ராஜம் கீதா, பட்டமேற்படிப்பு, மருத்துவ மாணவி, உயிர்வேதியியல் உயர்நிலைத் துறை, சென்னை மருத்துவக் கல்லூரி, சென்னை. மேற்கொள்ளும் ஆராய்ச்சியில் பங்கேற்க எந்தவித நிர்பந்தமின்றி, முழு சுதந்திரத்துடன் சுய நினைவுடன் முழு மனதுடன் சம்மதம் தெரிவிக்கின்றேன்.

இந்த ஆராய்ச்சி பற்றிய தகவல் தாளை பெற்றுக் கொண்டு அதன் முழு விவரங்களையும், நோக்கங்களையும் மருத்துவரின் மூலம் புரிந்துக்கொண்டு எனது மருத்துவ குறிப்புகள், பரிசோதனை முடிவுகள் மற்றும் இரத்தத்தை பயன்படுத்திக் கொள்ளவும் மேலும் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் எனவும் அச்செயலினால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்துக் கொண்டு முழு மனதுடன் சம்மதம் தெரிவிக்கின்றேன்.

எனது பரிசோதனை முடிவுகளை அறிவியல் சார்ந்த அமைப்புகள் மற்றும் மருத்துவ இதழ்களில் வெளியிடுவதற்கு முழு மனதுடன் சம்மதிக்கின்றனே.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்
/இடது கைவிரல் ரேகை

இடம் :

தேதி :

PROFORMA

Date : Sample Id : _____

Name : Age : Sex : Ht (cm): Wt (kg) :

Pre/Post Menopausal:

Ethnicity : Community : Duration of Symptoms/diseases

Treatment history :

Pregnancy □ Lactation □

Other Autoimmune Diseases : _____ if any duration _____

Associated diseases with duration :

Renal Failure □ PCOS □ Hypertension □

Ischaemic heart disease □ Tuberculosis □ Diabetes Mellitus □

Hypothyrodism □ others □

Drug Intake:

Statins □

Any other medications _____

Smoking :_____Passive Smoking :_____ Alternative Medicine intake:_____

Clinically :

1. 2 / ↑ swollen joints : _____
2. Morning stiffness lasting more than 1hr for atleast 2 weeks : _____
3. RF : Positive / Negative
4. Symptoms of hypothyroidism:
5. Blood Pressure:

BMI :

DAS 28 (3) :

Sample Collection : Date _____ Time _____

Sample Analysis : Date _____

Investigations :

Hemoglobin :

CRP :

Sr.Leptin :

Sr.Fasting Lipid Profile :

ESR :

Rheumatoid Factor :

DAS 28(3)

JOINTS		LEFT		RIGHT	
		Swollen	Tender	Swollen	Tender
Shoulder					
Elbow					
Wrist					
MCP	**1**				
	2				
	3				
	4				
	5				
PIP	**1**				
	2				
	3				
	4				
	5				
Knee					
SUB TOTAL					

Total Swollen Joints :

Total Tender Joints :

DAS 28 (3)Score	Activity
< 3.2	Low
3.2 to 5.1	Moderate
> 5.1	High

ACR CRITERIA 2010 – POINTS

- 1 large joint - 0
- 2-10 large joint - 1
- 1-3 small joints with or without Large joint involvement - 2
- 4-10 small joints with or without Large joint – 3
- >10 joints with involvement of atleast 1 small joint - 5

□ Negative RF/ Negative ACPA - 0 □

□ Low positive RF/ Low positive ACPA - 2 □

□ High positive RF / High positive ACPA - 3 □

□ Elevated ESR / Elevated CRP - 1 □

□ Duration of arthritis six weeks or longer - 1 □

TOTAL POINTS : _____